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Life at the Molecular Level: An Understanding of Biology provides a comprehensive and accessible exploration of the molecular foundations of biology. The book delves into the intricate processes that occur at the molecular scale, emphasizing how these processes are essential to understanding the structure, function, and regulation of biological systems. It covers a wide range of topics, including the molecular components of cells, the mechanisms behind genetic inheritance, the role of proteins and enzymes, and the principles of metabolism. The text effectively bridges the gap between molecular biology and broader biological concepts, making it an invaluable resource for students and anyone interested in gaining a deeper understanding of life at its most fundamental level.

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Dr. Semanti Ghosh

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Editor

Dr. Semanti Ghosh
Assistant Professor, Department of Biotechnology, School of Life Sciences,
Swami Vivekananda University, Barrackpore, West Bengal, India

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Content

Chapter - 1
Recent Advances in Archaebacteria Mediated Hydrocarbon Biodegradation in Water: A Concise Review

Authors

Raju Bhunia
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barrackpore, Barasat, Kolkata, West Bengal, India

Raj Manna
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barrackpore, Barasat, Kolkata, West Bengal, India

Subhashis Sarkar
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barrackpore, Barasat, Kolkata, West Bengal, India

Suranjana Sarkar
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barrackpore, Barasat, Kolkata, West Bengal, India

Bidisha Ghosh
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barrackpore, Barasat, Kolkata, West Bengal, India

Chapter - 1

Recent Advances in Archaebacteria Mediated Hydrocarbon Biodegradation in Water: A Concise Review

Raju Bhunia, Raj Manna, Subhashis Sarkar, Suranjana Sarkar and Bidisha Ghosh

Abstract

An era of increasing economic growth and human density presents expanding environmental challenges, hydrocarbons and petroleum waste are polluting soil, land for agriculture, and water bodies more and more. Ecosystems are seriously threatened by hydrocarbon pollution in aquatic environments also in soil, and conventional bacterial biodegradation techniques frequently break down under adverse circumstances. There are some hydrocarbon content pollutants are coal, oil, gasoline, hydraulic fluids that affects various ecosystems and also the human health. In current studies we can see the capability of archaea bacteria, or archaea to degrade of hydrocarbon. Harsh environment like- deep sea, hypersaline, extreme temperature and anaerobic ecosystem where normal bacteria are unable to survive, archaea operate without a hitch. The archaea produce methane through the process of methanogenesis by using hydrocarbon in anaerobic environment. Research has revealed the essential enzymes for the anaerobic breakdown of hydrocarbon, the key-enzymes are benzyl succinate synthase and alkyl-coenzyme M-reductase. Thermophiles and halophils, which are mainly extremophilic archaea, has a potential to breakdown petroleum hydrocarbon in high saline concentration and intense temperature. In addition, it has been shown that the groups of bacteria and archaea mainly sulfur-reducting bacteria and methanogens speed-up the breakdown of complex hydrocarbon. The latest study emphasizes the future possibilities of archaea in bioremediation approaches, especially when it comes to hard environment oil spill clean-up. The prospective application of archaea-mediated biodegradation that could improve the efficiency of hydrocarbon pollution control in aquatic environments is pointed out in this review, exposing up opportunities for other biotechnological purpose.

Keywords: Anaerobic breakdown, biodegradation, bioremediation, ecosystem, hydrocarbons pollution

1. Introduction

The global economy of today depends on fossil fuels, particularly coal and petroleum, to produce energy. 2020 saw a 0.9 million barrel per day growth in petroleum consumption and a historic 100 million barrels demand for liquid fuels (Radice *et al*., 2023). The aquatic world habitat hydrocarbon pollution is a very serious threat to the environment with several causes. Among the worst recognizable sources are oil spills, which frequently happen as a result of crashes involving pipelines, offshore drilling rigs, and oil tankers. Huge quantities of crude oil are spilled into the ocean, disrupting the aquatic ecosystems rapidly along with over time. For example, millions of gallons of oil leaked into the Gulf of Mexico during the Deepwater Horizon disaster in 2010, seriously affecting marine life. Another major factor of hydrocarbon pollution is industrial runoff. Water bodies in the vicinity are frequently exposed to hydrocarbon-containing wastewater from refineries, factories, and other industrial establishments. A wide range of hydrocarbons, from straightforward alkanes to intricate polycyclic aromatic hydrocarbons (PAHs), they are renowned for being poisonous and persistent. Multiple aromatic rings are fused together to generate PAHs, which are classified as non-soluble asphaltenes or soluble resins like pyrene, phenanthrene, and anthracene (Ossai *et al*., 2020). Another major factor in the pollution caused by hydrocarbons is urban runoff. Hydrocarbons from cars, asphalt, and other sources grow over surfaces in urban areas and get flushed into rivers, lakes, and seas during rainy seasons. Although this kind of pollution can frequently less obvious than oil spills, it can still be just as detrimental to aquatic life. The simplest hydrocarbons found in nature, methane, ethane, and propane, are typically found in gaseous form, while heavier hydrocarbons are found in solid or liquid forms. But this can differ depending on the oil field (Hsu *et al*., 2017). Among the hydrocarbons in the liquid state are aromatic compounds. The most well-known BTEX chemicals (benzene, toluene, and ethylbenzene are also the most poisonous and xylene) can constitute up to 60% of the petroleum's light fraction in cases when they don't possess substituents within their chemistry, both in the richer fraction where they one or more linked cycloalkane rings or substituents of alkyl (Heibati *et al*., 2017).

So, the hydrocarbon pollution is a very critical issue for our environment, specifically for aquatic ecosystem. Recent studies shown that we can control the hydrocarbon pollution by archaebacteria. A class of microorganisms different from bacteria and eukaryotes is called archaebacteria, or archaea. They are renowned for their capacity to flourish in harsh settings, including high salinity, high temperatures, and acidic or alkaline conditions. The

identification of extremophilic characteristics in archaea has broadened our knowledge of the variety and potential of microbiological life. For instance, hot springs, vents of hydrothermal energy, and other geothermal settings have been shown to include thermophilic archaea, which are obligate hyperthermophiles. Salt lakes, salt mines, and other hypersaline ecosystems have been shown to harbour halophilic archaea, which are thriving under high salinity conditions. It has been shown that drainage from acid mines, soda lakes, and other extreme pH settings are home to acidophilic and alkaliphilic archaea, which are found to flourish in alkaline and alkaline environments, respectively. The methyl-coenzyme M reductase (MCR), which promotes the final step in the anaerobic degradation of methane and is found in methanogenic archaea, which produce methane as a metabolic by product, is one example of an archaeal enzyme involved in hydrocarbon degradation. Another example is the benzyl succinate synthase (BSS), which triggers the initial step in the anaerobic degradation of toluene as well as other aromatic hydrocarbons and is found in sulfate-reducing and nitrate-reducing archaea, which use sulfate or nitrate as a resource.

Compared to obligatory bacteria, facultative anaerobic archaebacteria are more adaptive to environmental changes (Cason *et al*., 2019). In this review we focused on the some advanced archaebacteria (like- *Methanosaeta ssp*., *Haloferax sp*.) which are helps in hydrocarbon degradation. By archaea we can remove pollutants or contaminant from water bodies. Some applications of archaebacteria mediated hydrocarbon biodegradation will give some positive effects in future.

2. Mechanisms of hydrocarbon degradation by archaebacteria

2.1 Archaebacteria metabolism and energy pathways

In terms of cellular structure and biochemistry, archaebacteria, also known as archaea, are a distinct class of microorganisms that diverge greatly from bacteria and eukaryotes. The makeup of archaea's cell membranes is one of their most unique characteristics. The membranes of archaea are constructed of ether-linked lipids having isoprenoid chains, in contrast to bacteria, that contain a lipid bilayer made of fatty acids. Greater stability and tolerance to harsh conditions, such as high temperatures, high salinities, and acidic or alkaline environments, are offered by this special lipid composition.

Additionally, archaea have distinct cell wall architectures. Archaeal cell walls can be constructed of proteins, glycoproteins, polysaccharides, or pseudopeptidoglycan, but bacterial cell walls are normally composed of peptidoglycan. The capacity of archaea to flourish in harsh conditions is

facilitated by these changes. Take thermophilic archaea, which are able to withstand high temperatures and contain proteins and enzymes that are sustainable in hot conditions. Because they live in extremely saline settings, halophilic archaea have adapted to withstand high salt concentrations by maintaining their osmotic equilibrium and enzyme function. Clostridium, Zymomonas, Klebsiella, Enterobacter, and the archaeon Methanobacterium are the most often used bacterial strains (Nwidee *et al*., 2016). However, the bioremediation implemented by archaea is a complex mechanism that requires numerous steps and cooperation between different species capable of acting on hydrocarbons synergistically. Furthermore, it must be considered that there are numerous factors such as temperature and nutrient concentration that play a fundamental role in the remediation process (Kebede *et al*., 2021). For instance, under anaerobic circumstances, certain archaeal species can break down methane through a process called reverse methanogenesis. In this technique, many terminal electron acceptors are used (Truskewycz *et al*., 2019). The anaerobic methanotrophic archaea might employ methyl-coenzyme M reductase as a major enzyme, taking advantage of its reverse reaction, even if the metabolic pathway is not fully understood. Methane can be attacked by the bacteria Methylomirabilis oxyfera in addition to the archaea domain. It has the ability to activate methane monooxygenases by converting nitrogen oxides (NO) from decreased nitrite into N_2 and O_2 (Fan *et al*., 2012).

2.2 Enzymatic Systems Involved in Hydrocarbon Degradation

Archaea have a wide range of enzymes, which can effectively break down hydrocarbons. These enzymes, which each have a distinct function in the degradation of hydrocarbon molecules, includes oxygenases, alkane hydroxylases, and dehydrogenases. An enzyme class known as oxygenases adds oxygen atoms to organic substrates. Monooxygenases and dioxygenases (Alegbeleye *et al*., 2017), among other oxygenases, are essential for the first stimulation of hydrocarbon molecules in the context of hydrocarbon breakdown. One oxygen atom is incorporated into the substrate by monooxygenases, which also catalyze the reduction of the remaining oxygen atom to water. In contrast, dioxygenases integrate both oxygen atoms to the substrate. Toluene dioxygenase, for instance, catalyzes the transformation of toluene into cis-toluene dihydrodiol, that is further broken down into catechol and, ultimately, carbon dioxide and water. Based on what is known about bacteria that break down polycyclic aromatic hydrocarbons (PAHs), it was hypothesized that archaea, which are aerobic, could also break down PAHs using dioxygenases. This hypothesis focused on lipoxygenases (LOXs), which break down PAHs by oxidizing them by introducing two oxygen atoms, which

causes the aromatic ring to rupture through ortho- or meta-cleavages (Ghodrati *et al*., 2021). However, because of the hydrocarbons' large size, the metabolism of PAHs is more intricate. The primary genes implicated include doxycycline-inducible system (dox) (Lu *et al*., 2021), naphthalene dioxygenase (ndo) (Lee *et al*., 2018), and naphthalene dioxygenase (nah) genes (Singh *et al*., 2022). Monooxygenases called alkane hydroxylases catalyze the conversion of alkanes into alcohols. They interact with a broad variety of alkane substrates, including hydrocarbons with short and long chains. The initial stage of alkanes' aerobic destruction is called hydroxylation; this produces alcohols, which are further oxidized to produce aldehydes and carboxylic acids. Many hydrocarbon-degrading archaea involve the medium-chain alkane degradation process, which is facilitated by the alkane hydroxylase encoded by the alkB gene (Mcgenity *et al*., 2012). By moving electrons from organic substrates to electron acceptors like NAD+ or FAD, dehydrogenases catalyze the oxidation of organic substrates.

They are essential in the hydrocarbon breakdown process because they oxidize alcohols to produce aldehydes and carboxylic acids. Alcohols are converted to aldehydes by alcohol dehydrogenase, while aldehydes are converted to carboxylic acids by aldehyde dehydrogenase. The full oxidation of hydrocarbons to carbon dioxide and water depends on these processes. Co-factors' Assistance with Metabolic Efficiency refers to Non-protein substances known as co-factors help enzymes catalyze biological events. Cofactors such as NAD+, FAD, and coenzyme A improve the efficiency of the metabolic pathway in hydrocarbon breakdown. In redox processes, the electron carriers FAD (flavin adenine dinucleotide) and NAD+ (nicotinamide adenine dinucleotide) are involved. Dehydrogenase activity depends on them to transport electrons via the building block to NAD+ or FAD, where they create NADH or $FADH_2$ (Alaidaroos, 2023). By contributing electrons to the electron mobility chain, these decreased co-factors enable oxidative phosphorylation, which produces ATP. For the maintenance of electron flow and general metabolic efficiency, NAD+ and FAD availability is essential.

2.3 Factors Influencing Biodegradation Efficiency

The hydrocarbon breakdown efficiency of archaea is contingent upon several environmental conditions, such as pH, salinity, and temperature. Because of their reputation for thriving in harsh environments (Mohr *et al*., 2018), archaea's capacity for biodegradation is greatly influenced by its aversion to these conditions. One important element influencing the stability and activity of the enzymes that regulate hydrocarbon breakdown is temperature. Enzymes that are stable and active at temperatures above 60°C

are found in thermophilic archaea (Reysenbach *et al*., 2020), which are organisms that flourish at high temperatures. Because these enzymes are frequently more stable and catalytically efficient than their mesophilic counterparts, thermophilic archaea are a good fit for biological remediation in hot environments like oil reservoirs and geothermal springs. For instance, it has been demonstrated that the thermophilic archaeon Thermococcus can break down alkanes at temperatures as high as 80^0C (Dombrowski *et al*., 2018).

Another significant element that affects how well archaea biodegrade is salinity. Because they live in extremely saline settings, halophilic archaea have adapted to withstand high salt concentrations by maintaining osmotic balance and enzyme function. Among these changes include the build-up of suitable solutes, including potassium ions, and the existence of enzymes that can withstand salt. It has been demonstrated that halophilic archaea, such Halobacterium salinarum, can break down hydrocarbons in hypersaline settings, which makes them useful for bioremediation in salt lakes, salty mines, and other high-salinity settings (Mara *et al*, 2023). Environment's pH has an impact on the stability and activity of the enzymes responsible for hydrocarbon breakdown. Archaea that are acidophilic or alkaliphilic have adapted to live in acidic or alkaline conditions, respectively. The existence of pH-stable enzymes and processes is one of these adaptations.

3. Recent advances in archaebacteria-mediated hydrocarbon biodegradation

3.1 Newly Discovered Archaebacterial Strains

New strains of archaea have been identified through recent developments in microbiology, and they show promise for hydrocarbon biodegradation. Methanotrophic bacteria utilise methane, the most basic hydrocarbon, as a source of carbon and energy (Alaidaroos, 2023). Methanococcus and Methanosarcina are two examples of methanogens that have demonstrated the capacity to break down heterocyclic and aromatic hydrocarbons, on the other hand thermophiles such those in the genus Pyrococcus, are useful for bioremediation at geothermal settings since they have been connected to the breakdown of more complicated hydrocarbons at high temperatures. All the Aromatic molecules and longer aliphatic chains are left behind when the volatile hydrocarbons quickly evaporate (Alaidaroos, 2023). Bacterial chemotaxis can be influenced by hydrocarbons, which permits the germs to go toward contaminated locations (Logeshwaran *et al*., 2018). Industrial operations frequently result in halogenated hydrocarbons, which can be

degraded by certain strains like Halobacterium. These archaea are adapted to live in scenarios that would kill off many bacteria, and they flourish in high salinity settings. These strains have distinct metabolic pathways that allow them to use hydrocarbons as a carbon source. They are frequently isolated from harsh habitats including salt flats, oil fields, and deep-sea vents.

3.2 Genetic studies and genome sequencing efforts for understanding capabilities

To learn more about these recently identified archaebacteria's capacity for biodegradation, ongoing genetic research and genome sequencing are essential. These archaea's entire genomes have been easier to characterize thanks to high-throughput sequencing technology. There are some gene which help in degradation of hydrocarbons. AlkB, acmAB, prmABCD, and ladA are the genes encoding the enzymes involved in the peripheral metabolic processes for aliphatic hydrocarbons. Alkane 1-monooxygenase, an essential membrane enzyme that starts the hydroxylation of n-alkanes (C5-C12) to generate 1-alkanols, is encoded by the alkB gene (Alaidaroos, 2023).The availability of 45 genomes that are either complete or nearly complete form cultivated archaeal species has not only sparked new research areas that go well beyond the archaeal community, but it has also provided a crucial framework for tracking the genomes of uncultivated archaea in complex communities.

4. Environmental applications

Archaebacteria have the ability to change their metabolic pathways in response to oxygen limitation and can flourish in an oxygen-rich environment (Zhang *et al*., 2021). Furthermore, facultative bacteria may be significantly more prevalent in environments with varying oxygen levels, such as the water column in lakes and seas (Haas *et al*., 2019). Due to their special metabolic abilities, archaebacterial-which are recognized for flourishing in harsh environments-show considerable promise for bioremediation of water systems. Their function in oxidizing methane in wetlands, eliminating heavy metals, lowering sulfate in drainage from acid mines, and dehalogenating organic contaminants in groundwater is illustrated by case studies. These procedures aid in reducing pollution in acidified habitats, metal-contaminated rivers, and oil spills. Because they can survive in severe environments, archaea are useful for treating different types of contamination in water and restoring the ecosystem.

Hydrocarbons are a serious hazard to ecosystem function and living things. Numerous plant and animal species have gone extinct as a result of

their widespread existence, which has had negative ecological effects (Nzila, 2018). These substances consist of several aquatic conditions, including freshwater, saltwater, and wastewater, binding to particles and sediments. Thus, benthic organisms such as fish, insects, and filter feeders filter suspended petroleum, which exposes them to hydrocarbons (Logeshwaran *et al*., 2018). The ability of archaebacteria to adapt to changing environments makes them useful for the breakdown of complicated hydrocarbon compounds into more basic types (Zhang *et al*., 2021). The iron-containing minerals found in sediments can be used by archaea as alternate electron acceptors for respiration as the oxygen content drops from surface to subterranean conditions (Han *et al*., 2020; Dong *et al*., 2022). Concerns regarding possible ecotoxicological hazards and long-term effects on ecosystem health are raised by the arrival of archaebacteria for bioremediation. Even though archaea are naturally existing and frequently flourish in harsh conditions, adding them into unfamiliar habitats, such freshwater or marine systems, may upset the communities of bacteria and food webs that are already there. Because of their specific metabolic niches, ecotoxicological studies indicate that archaea are less likely to compete with native species; yet, the long-term impacts of archaea remain unclear, especially with regard to interactions with contaminants and horizontal gene transfer. Optimizing bioremediation methods requires an understanding of this complex interaction between hydrocarbon variety and microbiological communities (Kebede *et al*., 2021; Abdel-Shafy *et al*., 2016). Archaea may have an impact on methane production and nutrient cycling in marine environments, which could change the equilibrium of greenhouse gasses. Their effects on organic matter degradation and metal cycling in freshwater systems may have a domino effect on biodiversity and water quality.

5. Conclusion and Future perspective

A promising area of environmental biotechnology is the investigation of archaebacteria as mediators for hydrocarbon biodegradation in aquatic conditions. Recently, the unique biochemical and genetic characteristics of archaebacterial-especially their extremophilic nature have made them attractive options for combating hydrocarbon pollution in unorthodox or harsh environments, like extremes of salinity, temperature, or pH, where conventional bacteria might struggle. These microbes have proven to have a remarkable ability to break down hydrocarbons because of their sturdy enzymatic systems, which include methane monooxygenases, and their special lipid membranes, which offer stability under harsh circumstances. Various archaea, including as Halobacterium, Methanococcus, and Sulfolobus

species, have demonstrated the ability to digest a broad spectrum of hydrocarbons, ranging from basic alkanes to intricate polycyclic aromatic hydrocarbons (PAHs), which are renowned for their challenging degradation. In anoxic conditions, where methanogenesis might happen as a terminal degradation pathway, recent studies have emphasized the combined role of archaea in microbial consortia, wherein they cooperate with other bacterial species to enhance the general efficacy of hydrocarbon degradation processes. Notwithstanding these developments, a number of obstacles still need to be overcome before archaebacteria can be widely used in bioremediation projects. Obstacles that need to be overcome include the low development rates of many archaebacteria in comparison to other hydrocarbon-degrading bacteria, the difficulties of growing these organisms in the lab and in the field, and our insufficient knowledge of their metabolic networks according to hydrocarbon stress. Furthermore, although archaea are becoming more adept at genetic manipulation, their knowledge still falls behind that of bacteria, which limits the development of strains with optimum biodegradative pathways.

With more multidisciplinary research and technical developments, archaebacteria-mediated hydrocarbon biodegradation has a bright future ahead of it. Synthetic biology advancements may make it possible to build archaebacteria with improved hydrocarbon-degrading skills by improving metabolic flux or inserting genes for particular degradation pathways that lead to the full mineralization of hydrocarbons. Archaebacteria could be cultivated and used on a wider scale more easily if more advanced bioreactor systems were created to replicate the harsh conditions in which they live. Moreover, the combination of transcriptomics, proteomics, and genomics will help scientists understand the precise molecular processes that archaebacteria use to break down hydrocarbons. This will open the door to the discovery of new enzymes and routes of metabolism that may be used in biotechnological settings. Archaebacteria are being investigated for the bioremediation of various pollutants, such as heavy metals and radioactive substances, especially in harsh conditions where few other organisms can live. These environmental applications may go beyond hydrocarbon contamination. In order to scale up archaebacteria-based bioremediation technologies and make sure they are commercially feasible, ecologically safe, and able to handle the worldwide difficulties of hydrocarbon pollution in marine and freshwater ecosystems, cooperation between researchers, industry, and governmental organizations will be essential. Furthermore, the nature and activity of the archaebacterial communities associated with biodegradation may be affected by changes in aquatic ecosystems brought about by climate change, which may have an

impact on the long-term viability of these bioremediation techniques. This is a topic of growing interest. In summary, the use of archaebacteria for hydrocarbon degradation symbolizes a cutting-edge strategy with revolutionary potential for environmental remediation, providing a robust and flexible tool for reducing one of the most common environmental pollutants, even though there is still much to learn and develop.

Conflict of interest

The authors have no conflict of interest.

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Chapter - 2
Novel Nanoparticle-Based Strategies to Fight Multidrug-Resistant Bacteria: Ecological Synthesis, Antimicrobial Performance, and In-Silico Understanding

Authors

Sayantani Manna
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, West Bengal, India

Debjit De
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, West Bengal, India

Chapter - 2

Novel Nanoparticle-Based Strategies to Fight Multidrug-Resistant Bacteria: Ecological Synthesis, Antimicrobial Performance, and In-Silico Understanding

Sayantani Manna and Debjit De

Abstract

Multidrug-resistant (MDR) microorganisms are recognized as a primary cause of chronic diseases and deaths worldwide and can cause a wide range of illnesses, such as **urinary tract infections (UTI),** pneumonia, bloodstream infections, and surgical site infections. SNPs were synthesized by traditional chemical procedures and HMGNPs utilized environmentally friendly methods for in-vitro experiments. These nanoparticles' antibacterial properties were assessed against MDR ***Klebsiella pneumoniae*** and ***Escherichia coli*** strains that were isolated from UTI patients. Their antibacterial activity was evaluated using a variety of assays, such as **disk diffusion**, **minimum inhibitory concentration (MIC)**, and time-kill experiments. Through **molecular docking** simulations method, possible modes of action and binding affinities between **HMGNPs** and **SNPs** and important bacterial proteins were revealed. **HMGNPs** and **SNPs** both had notable antibacterial action, but HMGNPs were more effective than SNPs. According to in-silico research, HMGNPs may work through a variety of methods, including rupturing bacterial cell membranes and suppressing vital enzyme functions. Examine environmentally friendly ways to produce **cadmium sulfide (CdS)** nanoparticles and incorporate them into nano biopolymers to combat UTI bacteria. Green nanoparticle synthesis produces nanoparticles in an environmentally friendly manner by employing plant extracts in place of the dangerous chemicals and unfavorable conditions usually associated with conventional processes. Here, **cadmium sulfide** nanoparticles were produced biologically by utilizing the reducing and stabilizing properties of compounds derived from plants. The process lessens the harmful impact of the nanoparticles on the environment while simultaneously increasing their biocompatibility. The generated CdS nanoparticles were incorporated into a nano biopolymer matrix to make a

composite material. This nano biopolymer serves as a supporting framework for improving the stability and bioavailability of the CdS nanoparticles. These findings underline the importance of combining in-vitro and in-silico approaches in the development of novel antimicrobial treatments and demonstrate the potential of HMGNPs as potent agents in the fight against MDR UTI bacteria.

Keywords: Green nanoparticles, synthetic nanoparticles, phytochemical, MIC, thin layer chromatography, PCR, bioinformatics, etc.

1. Introduction

Multi-drug resistance is an antimicrobial resistance shown by a species of microorganisms to at least one antimicrobial drug in three or more antimicrobial categories. This Multidrug-resistant (MDR) microorganism remains the greatest undertaking in public health care. The number of infections produced using such resistant traces is growing globally. This has been further complicated by the increasing appearance and spread of **multi-drug resistance (MDR).** The most prevalent type of bacterial infections are urinary tract infections **(UTIs)**. The rising incidence of antibiotic-resistant UTI pathogens has created an urgent need for alternative therapeutic strategies. In this context, the ingenious application of nanotechnology has gained considerable attention, particularly through the use of heavy metal-based green nanoparticles (NPs) and synthetic nanopolymers. The key advantages of using nanoparticles are to penetrate bacterial cell walls and deliver targeted treatments, potentially overcoming the limitations of traditional antibiotics.

Phytochemicals are used for the synthesis of green nanoparticles. Phytochemicals are a good choice for nanoparticles because they are derived from plants, making them biocompatible and less toxic compared to synthetic materials. Many phytochemicals have inherent antimicrobial properties, enhancing the nanoparticles' efficacy against microorganisms. Using phytochemicals reduces reliance on synthetic materials, making the nanoparticles more environmentally friendly and often inexpensive to extract and process, reducing production costs also Phytochemical-based nanoparticles can be designed to biodegrade, reducing environmental impact. Phytochemicals, including **plumbagin** (the metabolite of ***Plumbago capensis***), show potent antimicrobial activity and are currently used as an adjunctive therapy with nanoparticles. Plumbagin is a good preference for the synthesis of nanoparticles due to its antimicrobial properties, Antioxidant properties, Reducing agent, Capping agent, Biocompatibility, Solubility, and

Cost-effective. The enhanced antimicrobial activity of the nanoparticles along with their unique optical and electronic properties is attributed to this study by using cadmium sulfide (CdS) in combination with plumbagin. Cd has a high toxicity rate in the human body, use at the nanogram level does not affect much, and traditional use decreases the toxicity to a certain level, favorable for human consumption. Rather Cadmium has a high target specificity in drug delivery systems. The choice of sulfur is made because of its high solubility and high coordination number.

The bacterium ***Pseudomonas fluorescence*** synthesizes nanoparticles by a synthetic biological method. This Gram-negative bacterium has a versatile metabolism that allows it to synthesize extracellular supernatants that dissolve in a polymer to facilitate nanoparticle synthesis. This bacterium is used because it is biocompatible which is why they are safe for use in biological systems, it can produce fluorescent pigments, and heavy metal resistance, is easy to culture and manipulate, and is also able to be easily scaled up for a huge scale nanoparticle manufacturing. **Polyethylene glycol (PEG) and citric acid** are mixed with ***P. fluorescence*** to prepare the synthetic nanoparticle. Furthermore, polyethylene glycol (PEG) and citric acid are selected from literature for the synthesis process to stabilize the nanoparticles, due to their existence increasing stability/biocompatibility of the particles, which is essentially associated with an incentive in its response time within biological systems.

2. Reason for using UTI Pathogens

There are many reasons that UTI pathogens are hard to eradicate. Since they are gram-negative bacteria, their outer membrane barrier, the existence of multidrug efflux transporters, endogenous antimicrobial inactivation, and other factors contribute to their inherent resistance to numerous antibiotics. They can form biofilms, physically block the orifice tubes, and if they become resistant to commonly used antibiotics (or set of them), the speed with which resistance genes transfer means that novel strategies need even more thought than is already discussed widely.

3. Antibiotic resistance profiling of UTI Pathogen

Antimicrobial resistance takes place when microbes (like bacteria, viruses, fungi, and parasites) trade and adapt over the years, developing resistance to tablets designed to kill them. One of the maximum common styles of drug resistance is antibiotic resistance - when bacteria end up resistant to antibiotics. Pills like antibiotics are an important tool for contemporary medicine. Without them, habitual medical strategies, minor

accidents, and commonplace illnesses can come to be existence-threatening. However, it's not simply bacteria-fungi, viruses, and parasites additionally expand antimicrobial resistance. The result is that many tablets, including antibiotics, antivirals, antifungals and antiparasitic are getting less effective at treating ailments. Resistant microbes can live on and multiply. If they're handed on to different humans, animals, or the environment, resistant infections can spread unexpectedly. Octa disc is a circular disc that is divided into a total of 8 segments, each segment contains a different antibiotic. Some Octa discs have the following antibiotics.

Antibiotics symbol concentration

1) Amikacin AK 30 mg
2) Co-Trimaxazole COT 25 mg
3) Colistin CL 40 mg
4) Augmentin AMC 30 mg
5) Netillin NET 30 mg
6) Norfloxacin NX 10 mg
7) Ceftriaxome CTR 10 mg
8) Ciprofloxacin CIP 5 mg
9) Furazolidone FR 50 mg
10) Amoxicillin AMX 10 mg
11) Cefotaxime CTX 30 mg
12) Gentamycin GEN 10 mg

By this assay, one can understand that the UTI Pathogens are resistant to which antibiotics and proved that the UTI pathogens are multi-drug resistant.

4. Conducting the PCR to detect the antibiotic-resistant gene

PCR can be performed to detect antibiotic resistance genes by amplifying specific DNA sequences associated with resistance. One should first isolate the genomic DNA of the UTI pathogen and then run the PCR. Primer selection is the crucial step in this technique, design or select a primer that specifically binds to the target antibiotic resistance gene. When the PCR technique is done then run this sample on a gel to identify whether the antibiotic resistance genes are present or not. If the genes are present then a dark band is shown under the UV light and if not present then no band will show.

5. Methods of biogenic NP production

5.1 Plant-based synthesis of NPs

When it comes to obtaining a better yield, plant-assisted NP synthesis is more efficient than microbial synthesis. Many metabolites and biochemicals found in plants, such as polyphenols, can act as both reducing and stabilizing agents during the synthesis of biogenic NPs [Anirudh Singh, Pavan Kumar Gautam *et al.* 2020]. Plant-mediated NP synthesis is cost-effective and environmentally benign because it doesn't require harmful chemicals [Anirudh Singh, Pavan Kumar Gautam *et al* 2020]. Three types of plant-mediated NP synthesis exist: extracellular, intracellular, and phytochemical-mediated [Anirudh Singh, Pavan Kumar Gautam *et al* 2020].

Green synthesis of nanoparticles is a method for producing nanoparticles using biological components and natural extracts instead of harmful chemicals. This approach is considered safer and more efficient than other methods, and it's also more environmentally friendly.

Nanoscale metal synthesis is an interesting topic since nanoscale metals are widely employed in many fields, including engineering, medicine, and the environment. At gift, nanoscale metals are specially synthesized via chemical strategies that have unintended outcomes inclusive of environmental pollutants, large power intake, and ability fitness issues. In reaction to these demanding situations, inexperienced synthesis, which makes use of plant extracts in place of industrial chemical sellers to reduce steel ions, has evolved. Current advances in the novice synthesis of gold (Au NPs), silver (Ag NPs), palladium (Pd NPs), copper (Cu NPs), cadmium (Cd NPs), and iron and its oxide (Fe NPs) were assessed in this review. The primary results show how complex the seasonal and geographic distributions of plants are, as well as how inexperienced synthesis is limited by production time and area, poor yield, and low purity. But, thinking about modern-day environmental issues and pollutants related to chemical synthesis, green synthesis allows for developing potentialities and capacity applications.

For the synthesis of green nanoparticles, the phytochemicals are extracted from the Plumbago plant leaves, and perform several biochemical tests for identifying the presence of **carbohydrates**, **alkaloids, protein, starch, amino acids, and tannins**. After those tests, a **TLC** was performed to identify the high-fluorescence bioactive compound.

Plumbagin is mixed with the cadmium sulfide (CdS) which is less soluble less reactive and has a high coordination number of 6, hence, six sulfur atoms attach themselves and form CdS. Na2S was used as a stabilizing agent to help

stabilize these compounds at high temperatures. Thus, the green nanoparticles are formed.

5.2 NPs synthesized using bacteria

There are two methods for producing NPs with the assistance of microorganisms: extracellular and intracellular processes. Since there is no requirement for a downstream procedure to collect NPs from the organisms, extracellular synthesis of NPs has an advantage over the intracellular technique in terms of time savings [Anirudh Singh, Pavan Kumar Gautam *et al* 2020]. The reductase enzyme found inside bacteria's mobile catalyzes the conversion of metal ions into metallic nanoparticles.

Synthetic nanoparticles are man-made particles with diameters between 1-100 nanometres (nm), designed and engineered with specific properties and functions. Synthetic nanoparticles can be made from a variety of materials, including Metals (e.g., gold, silver, iron), Polymers (e.g., polystyrene, polyethylene), etc. These nanoparticles have unique properties, such as high surface area-to-volume ratio, enhanced reactivity and stability, ability to target specific cells or tissues, and improved performance in various applications.

For the preparation of Synthetic nanoparticles such as the supernatant of ***Pseudomonas fluorescence*** was analyzed and mixed with a synthetic polymer which is **PEG- Citric acid**. Pseudomonas fluorescens is a Gram-negative, rod-shaped bacterium that is commonly found in soil, water, and plant surfaces. It's known for its ability to fluoresce under UV light due to the production of a pigment called pyoverdine. This bacterium plays a role in the biocontrol of plant pathogens, and bioremediation of environmental pollutants, and can be used in various industrial applications. It’s also studied for its potential in agricultural and environmental science.

6. Indication of nanoparticle formation

The formation of nanoparticles is typically indicated by several key physical and chemical changes. These include visual cues such as a color change or increased turbidity in the solution due to the scattering of light by the particles. Analytical techniques like UV-visible spectroscopy can reveal characteristic absorption peaks, while dynamic light scattering (DLS) provides information on particle size distribution [Y Georgalis, p. Umbach *et al* 1997]. Structural confirmation can be obtained through transmission electron microscopy (TEM) and X-ray diffraction (XRD), which offer insights into particle morphology and crystallinity. Additional methods like zeta potential and Fourier-transform infrared spectroscopy (FTIR) [researchgate.net] assess the stability and surface chemistry of nanoparticles.

Together, these indicators provide a comprehensive understanding of nanoparticle formation.

- **Colour change:** In many cases, a solution changes color when nanoparticles form due to surface plasmon resonance. For example, when the polymer of PEG- Citric acid was mixed with the sup of ***Pseudomonas fluorescence*** and formed the nanoparticles then the fluorescence decreased. It can be inferred that the decrease in fluorescence is due to the efficient binding of the PEG Citric acid polymer with the bioactive components present in the sup.
- **Turbidity or opacity:** The appearance of turbidity or cloudiness in a previously clear solution is often a sign of nanoparticle formation, as the particles scatter light.
- **UV-Visible spectroscopy:** A distinct absorption peak, often in the visible range (for metal nanoparticles like gold and cadmium), is a common signature of nanoparticle formation.

For example,

The cut wavelength* of CdS in natural form is 525nm

So, E = hν/ʎ; E = 1240/525 = 2.36 eV

When Plumbagin and CdS form the nanoparticle then the cut wavelength of CdS is around 462 nm.

So, E = hν/ʎ; E = 1240/462 (from the graph) = 2.68 eV

As the ʎ is decreasing the energy (E) is increased; and since the energy is increased, the fluorescence property may decrease.

The minimum wavelength of light which causes electrons to be emitted from a metal surface

7. Variables affecting the synthesis of different NPs

7.1 Temperature

A significant amount of research is being conducted globally to investigate temperature regulation over nanoparticles. One of the biggest influencing elements on the morphology-the size, shape, and charge of synthesis of the NPs-is temperature. Temperature affects the size, production, and several forms of nanoparticles (NPs), including spherical, rod-like, octahedral, and triangle platelets. Response charge and the creation of nucleation centers both increases with temperature, although slower nucleation at lower temperatures may lead to smaller particles. The formation

of crystalline or amorphous nanoparticles, which influence their ultimate characteristics, is also influenced by temperature. The size, shape, and structure of nanoparticles can be precisely tailored for certain uses by adjusting their temperature.

The results showed that while the NPs' yield was found to be higher, their scale also decreased as the temperature increased. Scanning electron micrographs of NPs provided sufficient evidence to substantiate this reality. Using a biocompatible polymer PEG, Fleitas-Salazar *et al*. (2017) investigated the effects of temperature and an oxidizing environment on the production of Ag NPs. According to the study, PEG's ability to decrease silver salt at 100°C was excessive. They deduced that at 100°C, the functional groups of PEG engaged in active interactions with Ag molecules, leading to the production of more stable Ag nanoparticles. Ag ions were discounted at 60°C due to the hydroxyl agencies in PEG being oxidized. The authors concluded that there are unique processes for Ag NP synthesis at different temperatures as a result.

7.2 pH

The production of NPs is significantly influenced by the pH of the response. pH controls nucleation center formation in addition to temperature. A wider range of nucleation sites are created as pH rises, which increases the amount of metallic NPs that are formed. It has been established that pH plays a crucial role in determining the length and shape of the NPs. In 2004, Armendariz *et al*. investigated the production of Au NPs from A. sativa at various pH levels. It was shown that while there were comparatively fewer NPs generated at lower pH levels (pH 2), the size of the NPs was noticeably greater (2585 nm). They proposed that Au NPs combine to generate big-sized NPs because they stop forming new nucleation centers at lower pH values. In contrast, tiny-sized NPs were generated at a slightly higher pH (pH 3-4).

7.3 Reaction time

Reaction time was found to be one of the key variables that affect the shape of NPs, along with temperature and pH. Karade *et al*. (2018) conducted a noteworthy study to examine the impact of reaction time on magnetic nanoparticles [48]. In response to the application of green tea extract, Fe NPs have been produced from ferric nitrate. It was discovered that the reaction time affects both the structural and magnetic properties of magnetic nanoparticles. The particle size increases from 7.5 nm to 12 nm with the increase in reaction time. Additionally, it was noted that an increase in response time may improve the NPs' saturation magnetization (Ms).

8. The efficacy of the nanoparticles against UTI Pathogen

Microbes affixed to surfaces, known as biofilms, can be found in industrial, medicinal, and environmental contexts. In actuality, living in a biofilm most likely signifies the predominant form of growth for bacteria in optimal conditions. A few unique characteristics characterize mature biofilms. Typically, an extracellular matrix enveloping biofilm microorganism provides structure and defense for the network. Even the functional architecture of microbes growing in a biofilm is typically composed of macro colonies, or large numbers of cells, encircled by channels filled with fluid. The resilience of biofilm-grown microorganisms to numerous antimicrobial agents, including therapeutically important medicines, is another well-known characteristic of these organisms.

The microtiter dish assay, which has been used primarily to study bacterial biofilms but has also been used to study fungal biofilm formation, is an essential tool for monitoring the initial stages of biofilm formation. The assay's use of static, batch-growth conditions prevents the development of mature biofilms that are typically associated with more mobile structures.

This easy microtiter dish assay allows for the formation of a biofilm on the wall and/or backside of a microtiter dish. The excessive throughput nature of the assay makes it beneficial for genetic monitors, in addition to testing biofilm formation by a couple of traces under diverse growth situations.

8.1 Biofilm eradication

Biofilm eradication involves disrupting the protective extracellular matrix surrounding bacterial communities, which enhances the penetration and effectiveness of antimicrobial agents. The procedure is mentioned below:

- **Initial attachment:** Initially reversible, this stage disrupts weak bonds attaching bacteria to surfaces.
- **Irreversible attachment:** Flagella degrade or are replaced by cilia, stabilizing the attachment.
- **Initial maturation:** Involves quorum sensing, where bacteria communicate via signaling molecules, regulating biofilm formation, virulence, and gene expression. Targeting quorum sensing and exopolysaccharide (EPS) attachment is crucial for biofilm eradication, as EPS plays a key role in biofilm development.
- **Final maturation:** Tower formation in the cup plate assay indicates biofilm development, with bacterial colonies growing vertically from

the agar surface to the air-liquid interface. This maturation is influenced by factors like quorum sensing and EPS production.

- **Cell Dispersion:** Individual or small clusters of cells detach from the biofilm and disperse into the surrounding environment, often regulated by molecular mechanisms triggered by environmental signals or quorum sensing, enabling colonization of new sites or adaptation to changing conditions.

After 72 hours of biofilm formation, the modulators or nanoparticles are added and incubated for 24 hours. A series of steps are done and stain the biofilms with crystal violet and measure the optical density to quantify the biomass of the biofilm. OD value low means the biofilms are eradicated by the nanoparticles.

This standardized approach allows for the evaluation of different modulators and combinations in their efficacy against biofilm formation and eradication, providing valuable insights into potential antimicrobial strategies.

8.2 Antibiofilm assay

An anti-biofilm assay is a laboratory approach used to evaluate the ability of a substance (such as an antimicrobial agent) to prevent the formation of biofilms. In the process of antibiofilm assay, the nanoparticles are first added to the well and then add bacterial culture, allowing the bacteria to form the biofilm. But in the presence of nanoparticles which act as an antibiotic, the bacteria are unable to form the biofilm. Measure the biomass of the biofilm by measuring the OD value.

8.3 MIC (Minimum Inhibitory Concentration)

It is used to estimate the antimicrobial efficacy of synthesized nanoparticles. The MIC allows the determination of the minimum concentration of test suspension that allows the complete of inhibition of microbial growth. It is effective against both gram-Ve and gram +Ve bacteria. A variety of test suspensions can be used to estimate their MIC against the pathogens.

Which nanoparticles are capable of controlling microbial growth at the lowest concentration, those nanoparticles are considered the most effective antibiotic.

8.4 TTC assay (2,3,5- Triphenyl- Tetrazolium Chloride)

Triphenyl tetrazolium chloride (TTC), or tetrazolium chloride (with the system 2,3,5- triphenyl-2H-tetrazolium chloride) is a redox indicator generally

utilized in biochemical experiments in particular to indicate mobile breathing. Within the TTC assay (also known as TTC test or tetrazolium test), TTC is used to differentiate between metabolically lively and inactive tissues. This dye is colorless inside the oxidized form and adjustments to crimson while reduced using microorganisms, due to the formation of formazan. Stay microorganisms, for instance, E. coli and other coliforms reduce TTC with the aid of enzymatic action, forming crimson coloration formazan that is saved inner granules in the cells. TTC is fairly advocated in trying out pasteurized milk because there may be an excessive percentage of microorganisms not able to lessen TTC in pasteurized milk, which can't be detected by using laboratory strategies. Higher concentrations of TTC may also have an inhibitory effect on the increase of Gram-effective organisms.

The evaluation of TTC Assay discovered that the elements had been able to inhibit the formation of formazan. The smaller the quantity of formazan fashioned higher the factor.

8.5 Efficiency of nanoparticles on MDR UTI Pathogen by cup-plate method

Cup plate assay is a type of microbiological assay or we can say microbial assay. It is a type of assay in which the relative potency or activity of a compound is estimated by measuring the amount that is required for producing a suitable effect on our test organisms under suitable conditions. It is also called the cylindrical plate method. It involves the diffusion of the compound throughout the agar and preventing the growth of microbes producing a clear zone. The diameter of the zone is measured to estimate the antimicrobial property, more the diameter of the clear zone, the more antibacterial properties of the compounds.

9. Computer-based drug designing or Molecular docking dry lab-based techniques or in silico techniques

The study of how two or more molecular systems (such as a medication and an enzyme or protein) pair with one another is known as molecular docking. A molecular modeling technique called docking is used to predict the interactions between proteins, enzymes, and tiny molecules, or ligands. A protein's dynamics are largely determined by its capacity to interact with smaller molecules to form supramolecular complexes, which can also either enhance or hinder an enzyme's organic characteristics. Molecular docking describes the behavior of tiny molecules inside the binding pocket of target proteins. The method aims to identify the precise positions of ligands within a protein's binding pocket and to predict the ligand-protein affinity. Protein-

small molecule (ligand) docking, Protein-nucleic acid docking, and Protein-protein docking are the three types of docking that are entirely dependent on the types of ligands.

Molecular docking is used because we want to understand the efficacy of our nanoparticles in opposition to the DNA and Protein of the pathogen or really, we want to recognize the mode of movement of our synthesized nanoparticles.

The bacterial protein FtSz can be used to estimate the impact of NP. Uses the bacterial protein FtsZ for the experiment since it is involved in cell division and forms single-stranded filaments through polymerization dependent on guanosine 5'-triphosphate (GTP) [Francesca Ruggieri, Nina Compagne *et al* 2023]. These FtsZ filaments create the Z-ring, a cytokinetic ring, in the mid-cell, where they can draw in additional essential components for the synthesis of peptidoglycan and the creation of septa, both of which trigger cell division [Francesca Ruggieri, Nina Compagne, *et al*., 2023]. Since FtsZ is crucial to the bacterium and is extensively conserved across bacteria, it possesses all the desirable characteristics of a novel target for antibiotic drugs [Francesca Ruggieri, Nina Compagne *et al* 2023].

The negative binding energy confirms that the binding of the ligand to the target macromolecule is a spontaneous reaction. It can be concluded that the nanoparticle has more affinity for the protein molecule and binds readily to it which might hamper its normal structural and functional capability. The inhibition of FtSz assembly restrains the cell division complex known as divisome which results in filamentation leading to cell lysis [Swayansiddha Tripathy *et al* 2019].

10. Conclusion

The study of in vitro and in silico analysis of heavy metal green nanoparticles and synthetic nanoparticles has yielded significant insights into their efficacy against multidrug-resistant (MDR) urinary tract infection (UTI) pathogens. The following key conclusions can be drawn from the study:

- **Enhanced Antimicrobial Activity**

Both green and synthetic nanoparticles demonstrated potent antimicrobial activity against MDR UTI pathogens. However, green nanoparticles, synthesized using eco-friendly methods, exhibited slightly higher efficacy, possibly because of the presence of bioactive compounds that enhance their antimicrobial properties.

- **Mechanisms of Action**

The in-silico studies provided detailed insights into the mechanisms of action of these nanoparticles. Both types of nanoparticles were found to disrupt bacterial cell walls, generate reactive oxygen species (ROS), and interfere with essential cellular processes, leading to bacterial cell death. The molecular docking studies further confirmed strong interactions between nanoparticles and key bacterial proteins.

- **Comparative Analysis**

When comparing green nanoparticles to their synthetic counterparts, the green nanoparticles showed a more favorable safety profile with lower cytotoxicity toward human cells. This suggests their potential as safer alternatives for treating infections caused by MDR pathogens.

- **Synergistic Effects**

The study highlighted the potential of using nanoparticles in combination with traditional antibiotics. The synergistic effects observed in the in vitro experiment suggest that such combinations can significantly reduce the effective dose of antibiotics required, thereby mitigating the risk of further resistance development.

- **Environmental and Economic Benefits**

Green synthesis of nanoparticles not only offers an environmentally friendly approach but also reduces production costs. Utilizing plant extracts and other natural resources for nanoparticle synthesis is both sustainable and economically viable, promoting their application in large-scale production.

In conclusion, the in vitro and in silico investigations affirm the promising role of heavy metal green nanoparticles and synthetic nanoparticles as effective agents against MDR UTI pathogens. Their incorporation into existing treatment regimens may want to revolutionize the management of drug-resistant infections, emphasizing the significance of persistent studies and development in this area.

11. Future aspect

The promising results from the in vitro and in silico studies on the efficacy of heavy metal green nanoparticles and synthetic nanoparticles against MDR UTI pathogens pave the way for several future research and development directions:

- ***In Vivo* Studies**

Conducting extensive in vivo studies to assess the protection, bio-distribution, pharmacokinetics, and therapeutic efficacy of these nanoparticles in animal models. These studies will provide crucial data on the potential of nanoparticles to treat MDR UTI infections in real biological systems.

- **Clinical Trials**

Initiating clinical trials to assess the safety and effectiveness of nanoparticle-based treatments in humans. These trials will be instrumental in translating laboratory findings into viable clinical therapies, ensuring that nanoparticle formulations are safe, effective, and well-tolerated by patients.

- **Precision Targeting**

Nanoparticles can be engineered to target specific bacterial cells, reducing the collateral damage to beneficial microbiota and minimizing side effects.

- **Combination Therapies**

Investigating the potential of combining nanoparticles with existing antibiotics or other antimicrobial agents to enhance therapeutic efficacy and decrease the chance of resistance development. Synergistic effects can lower the required doses of conventional drugs, minimizing side effects and resistance.

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Chapter - 3
Oncolytic Viruses: Revolutionizing the Landscape of Cancer Therapies

Authors

Tanisha Sengupta
PG student Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Pritha Pal
Assistant Professor, Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Chapter - 3

Oncolytic Viruses: Revolutionizing the Landscape of Cancer Therapies

Tanisha Sengupta and Pritha Pal

Abstract

Cancer has been one of humanity's greatest foes since the beginning of time, making it a popular subject for medical research and treatment. Despite ongoing advancements, cancer treatment strategies-particularly oncolytic virus (OV) therapy-attracted far greater interest. Oncolytic viruses do have a lot of drawbacks, though, so research on combination therapies is being done to increase their effectiveness and give patients new hope. In order to fully utilise oncolytic virus therapy and get beyond the drawbacks of single-therapie-based treatment paradigms, clinical research is now refocusing on combination approaches. This combo strategy is based on the discovery that injecting oncolytic viruses causes local tumour inflammation in a favourable way, altering the immune system to enhance the effects of immunotherapy. By reducing the tumor's ability to evade the immune system, this mechanism enables the immune system to identify and combat cancers more quickly. In phase II studies for glioblastoma in Japan, G47Δ, a third-generation HSV-1 oncolytic virus, has been accorded "Sakigake" designation; regulatory bodies would give the treatment priority review and quick approval. This result will probably need to come from particular antitumor immunity, the expected long-term driver of extended patient survival that necessitates each virus to reproduce specifically within tumours, even though several oncolytic viruses are being tried in clinical research. Oncolytic virus therapy has the potential to become a common cancer treatment.

Keywords: Oncolytic virus, T-Vec, Combined therapy, limitations

Introduction

According to the International Agency for Research on Cancer (2019), cancer has become one of the top causes of mortality globally, with about 10 million deaths and 18 million new cases diagnosed each year. The incidence and mortality rates of cancer continue to climb despite significant progress in

cancer prevention and diagnosis (Lippman *et al*. 2009, Segovia-Siapco *et al*. 2019; Bray *et al*. 2020).Conventional cancer therapies, such as chemotherapy, radiation, and surgery, are linked to severe cytotoxicity and a host of adverse effects. They also lack specificity for tumour cells. Kaufman and colleagues (2015).Furthermore, classical immunotherapy still has a low success rate-between 10% and 30%-which emphasises the urgent need to increase its effectiveness. One of the most promising avenues for treating cancer among the different immunotherapy techniques is oncolytic virotherapy. This novel treatment makes use of viruses that specifically multiply within cancer cells and kill them. Following developments in immunotherapy, including immune checkpoint inhibitors, and conventional chemotherapy, oncolytic virotherapy holds the potential to completely transform the treatment of cancer. Oncolytic viruses are a revolutionary advancement in the fight against cancer because they selectively target and proliferate within cancer cells while sparing healthy tissues. They can be either naturally occurring or genetically modified.

Properties of oncolytic virus

- **Oncolytic properties:** Infected cells, whether naturally occurring or genetically modified, are the site of selective replication for oncolytic viruses (OVs). Viral clearance leaves normal cells unharmed. Viral replication causes tumour cells to lyse, which starts the induction of cell death pathways. New tumour cells are infected by the virus offspring released as a result of oncolysis.
- **Immuno-stimulatory properties:** Oncolysis, which results from virus replication, triggers the production of pathogen- and damage-associated molecular pattern molecules (PAMPs and DAMPs, respectively), as well as antigens specific to viruses and tumours. On the one hand, dendritic cells' subsequent absorption and presentation of antigens causes T lymphocytes that are specific to viruses and tumours to be induced. Dendritic cells, on the other hand, absorb these and use them to activate T cells that are specific to viruses and tumours. On the other hand, it triggers a local inflammatory response that results in the release of chemokines that draw in T cells. These chemokines attract T lymphocytes that are unique to the tumour and the virus to the location where they are required to perform their duties at the tumour site.
- **OVs as a transgene delivery platform:** In order to further activate an antitumor immune response, several OVs (adenovirus and vaccinia virus) can be modified to express exogenous genes (armed

oncolytic viruses), such as cytokines or antibodies, with specificity for the tumour microenvironment.

Mechanism of action

Lysis of tumor cell

In normal cells

In order to combat infection, normal host cells can eliminate viruses and initiate signalling pathways. Tumour cells, on the other hand, do not have this antiviral ability, which allows the viruses to grow inside of them. By selectively replicating and lysing cancer cells while sparing healthy ones, oncolytic viruses take advantage of this mechanism. Additionally, OVs release progeny virions that infect nearby uninfected tumour cells. Increasing their oncolytic impact viral infections trigger antiviral pathways in the majority of healthy cells, which prevent the virus from replicating. IFN and other important molecules, including jenus kinase (JAK), signal transducer and activator of transcription (STAT), interferon regulatory factor 9 (IFN9), and toll-like receptors (TLRs), are among the signalling cascades that are started upon virus recognition. This cascade leads to a transcriptional pathway that stops the virus from spreading and causes infected cells to undergo necrosis or apoptosis. By infecting the IFN receptor, the innate immune system-induced local synthesis of IFNs further increases the antiviral activity. Myeloid differentiation primary response protein (MYD88), adapter-containing TIR domain that induces IFN β, IRF7, IRF3, and nuclear factor-κB (NF-κB) are among the proteins that TLRs need to start signalling. Following their activation of the JAK STAT pathway, type I IFNs upregulate cell cycle regulators such as IRF7 and protein kinase R. By binding to viral particles and triggering type I IFN-mediated transcription, these regulators limit the propagation of viruses.

In cancer cells

However, cancer cells may have limited signalling pathways, which could hinder the clearance of viruses. Furthermore, a number of surface receptors, including CD46, ICAM-1 (CD54), DAF (CD-55), and integrins, may be expressed in high concentrations by cancer cells, which facilitates virus entry into tumour cells. Within the innate signalling system, cancer cells may downregulate important signalling molecules such as RIG-1, IRF-7, and IRF-3. Because the signalling molecules block the detection of viral particles, cancer cells are consequently more vulnerable to viral replication. In addition to decreasing the activity of the type I IFN signalling pathway, cancer cells may also limit the pro-apoptotic and cell-regulating properties of type I IFNs.

After the cancer cells rupture and killed, the viruses are continues to infect and eliminates other cancer cells (Tian Y *et al*.2022).

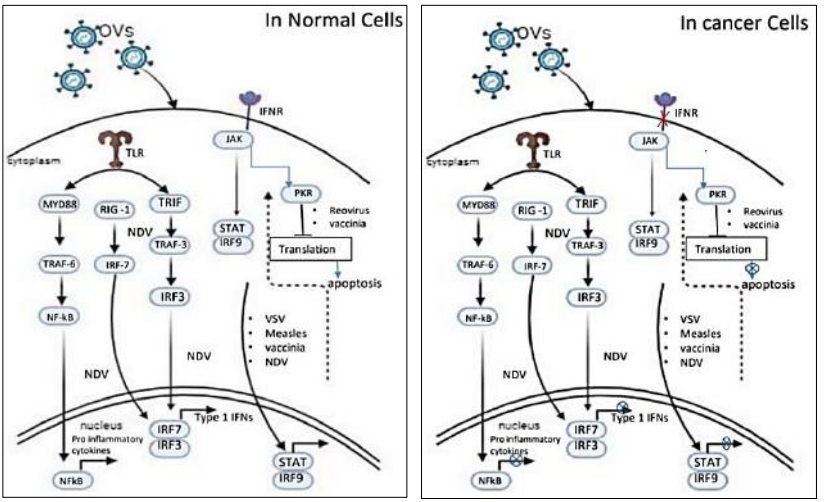

Fig 1: (a) Following viral infection, healthy cells activate an antiviral pathway. (b) In cancer cells, this process is impaired by the reduced expression of key components, increasing their susceptibility to viral replication. This image is modified from that in Kaufman, H.L. *et al*., Nat Rev Drug Discov, 2015

Immune responses

Following an OV-induced oncolytic cell, tumour cells produce a particular antigen that is linked to the tumour. OVs have the ability to trigger apoptosis, necrosis, and pyroptosis, among other types of immunogenic cell death (ICD) (Guo ZS *et al*. 2014). When a tumour cell lyses, pathogen-associated molecular patterns (PAMPs), like viral proteins and nucleic acids, and damage-associated molecular patterns (DAMPs) from dying host cells may be released. DAMPs, which promote the innate immune response, include cytokines like type I IFNs and IL-1 family, as well as nucleic acids including mtDNA, ATP, high mobility group box 1 protein (HMGB1), and heat shock protein (Ma J *et al*.2020, Hu L *et al*.2017). Pattern recognition receptors (PRRs), including stimulator of IFN genes (STING), Toll-like receptor (TLR) adaptor molecule 1 and TLR3 on NK cells and macrophages, detect these DAMPs and PAMPs (Shen Z *et al*.2023). By promoting the release of cytokines such type I interferons, tumour necrosis factor-α, and interleukin-12, this process starts a proinflammatory environment. These cytokines promote antigen-presenting cells' development, which aids in the adaptive immune response. APCs process and present the tumour antigens on

their MHC class I molecules. APCs have the ability to go to secondary lymphoid organs, like lymph nodes, where they engage in interactions with T lymphocytes. Tumor-specific cytotoxic T lymphocytes are activated and proliferate as a result of this interaction. Following chemokine guidance, activated CTLs move to the tumour location and identify tumour cells expressing the same antigen they were primed against (Ma R *et al*. 2023). After that, they release cytotoxic chemicals like granzymes and perforin, which cause tumour cells to die. Natural killer cells are essential in the fight against viral infections and tumours. MHC-I molecule expression is frequently downregulated by virus-infected cancer cells as a defence against cytotoxic T cell identification and destruction. However, NK cells, which use activating receptors to detect the absence or decreased levels of MHC-I on the cell surface, target them specifically because of this drop in MHC-I. Once activated, NK cells produce cytokines like IFN-γ and TNF-α and release cytotoxic chemicals like FAS_FAS ligand signalling to destroy tumour cells. These cytokines enhance the recruitment of immune cells to the tumour microenvironment by activating dendritic cells and reprogramming tumor-supporting M2 macrophages into proinflammatory M1 phenotypes. The innate immune system is boosted by this stimulation of NK cells and DCs, which also promotes the release of TNF-α, IFNs, IL-12, IL-16, and chemokines. Tumor-specific T-cells are essential for the adaptive immune response against tumours during oncolytic viral infections. Tumor-specific antigens are released by dying tumour cells and are picked up by APCs. After migrating to the lymph node, these cells provide the T cells the antigen, which triggers a strong adaptive immunological response. After entering the tumour tissue, these activated T cells-particularly CD8+T cells-seek for and eliminate tumour cells that have the same antigens (Hu JCC *et al*. 2006).

Types of oncolytic virus

The different tropism, structure and life cycle of different oncolytic viruses have implications in virotherapy. A wide range of viruses with diverse properties are under investigation clinically.

Table 1: Types of oncolytic virus and its modification for cancer therapy

Virus	Genome Size	Name	Cancer Type	Efficiency
HSV 1 (Herpes Simplex virus 1)	152kb linear double stranded DNA Genome	**T-vec:** The γ34.5 and α47 genes have been deleted in T-vec, a genetically altered form of the herpes simplex virus type 1 (HSV-1). The gene for human granulocyte-macrophage colony stimulating factor (GM-CSF) is introduced into the γ34.5 loci that have been removed. Eliminating this gene is essential for guaranteeing cancer-specific replication and lowering pathogenicity. Normally, during viral infection, the γ34.5 gene reverses the host cell's inhibition of protein production. The virus cannot replicate in healthy cells if this gene is disabled. However, because cancer cells do not show the same shut-off response, HSV-1 missing γ34.5 can still reproduce in them. (Markert JM *et al*. 2006, Agarwalla PK *et al*. 2012, Liu BL *et al*. 2003, He B *et al*. 1997, Chou J *et al*. 1992, and Hu JCC *et al*. 2006)	**T-vec:** metastatic melanoma	**T-Vec:** In phase-II trials, T-vec was assessed for patients with metastatic melanoma; however, no full or partial responses were noted. Overall, 26% of respondents in a single-arm phase-II experiment responded to both injected and non-injected lesioning, including visceral lesioning (Senzer NN *et al*. 2009).The medication received approval in the United States in 2015 and Europe in 2016.
		G47Δ: By inserting a second deletion mutation into the genome of G207, a second-generation HSV-1, Todo *et al*. produced G47Δ, also known as Teserpaturev, a third-generation oncolytic HSV-1.G47 Δ was developed mainly to enhance the ability to elicit specific antitumor immunity, with the aim of enhancing the antitumor efficacy while preserving the safety features of G207 (Mineta T *et al*. 1995; Todo T *et al*. 2001). Two of the G47 mutations occur in the γ34.5 and α47 genes, which are used by T-Vec. Furthermore, the ICP6 gene has been rendered inactive by the insertion of the Escherichia coli LacZ gene into G47Δ. The ICP6 gene encodes the main subunit of ribonucleotide reductase (RR), which is required for the synthesis of viral DNA. HSV-1	**G47Δ:** Glioblastoma (Cheema TA *et al*.2013)	**G47Δ:** Of the third generation HSV-1, only G47Δ has been tested on humans thus far. After the successful completion of the phase I–IIa study in patients with recurrent glioblastoma in Japan in 2014, a phase II investigation in patients with residual or recurrent glioblastoma started in 2015. In February 2016, the Japanese Ministry of Health, Labour, and Welfare (MHLW) designated G47 as a "Sakigake" breakthrough therapeutic drug. "Ahead of the world" is what the Japanese word "sakigake" signifies.

		replication may only be supported by quickly proliferating cells that express enough host DNA to compensate for the inadequate viral RR.		
Vaccina Virus	190kb linear double stranded DNA Genome	**JX594:** (pexastimogene devacirepvec, Pexa-Vec) It is a genetically modified vaccine virus that inserts the human GM-CSF gene to strengthen the immune response against tumours. Additionally, it contains a mutation in the TK gene that permits cancer cells to replicate selectively. The virus also has a lacZ gene, which enables cancer cells to replicate selectively. The virus also has a lacZ gene insertion in the TK region, which acts as a marker. Parato KA *et al*. (2012), Kim JH *et al*. (2006), and Kirn DH *et al*. (2009).	Advanced stage of Hepatocellular Carcinoma	In a phase I study, intralesional injection of primary or metastatic liver tumours producing JX-594 was generally well tolerated in the context of JX-594 replication, GM-CSF expression, and systemic dissemination. Direct hyperbilirubinemia was the dangerous side effect at the dosage limit (Breitbach CJ *et al*. 2011). To evaluate the feasibility of intravenous delivery, a phase I dose escalation experiment employing high dose JX-594 was carried out. A randomised phase II dose-finding study was conducted with patients with hepatocellular cancer.(Heo J. and others, 2019)
Adenovirus	26-46 kb linear double stranded DNA Genome	**CG0070:** The human GM-CSF gene was genetically engineered into the Ad5 adenovirus to create the oncolytic adenovirus known as CG0070 (Ramesh *et al*. 2006). This allowed the human E2F-1 promoter to drive the E1A gene in bladder cancer, where the binding of the tumour suppressor protein Rb produced a transcriptionally active E2F-1 (Zwicker J *et al*. 1995).	Bladder cancer	CG0070 is being administered intravenously treating patients with BCG-refractory non-muscle-invasive bladder cancer at a dose of 1012 vp per week for six weeks as part of a single-arm phase-III trial. The same induction cycle is continued every six months for patients who have a partial or full response six months following the first course of treatment (Burke JM *et al*. 2012).
Reovirus	23.5kb with double stranded RNA Genome	**Reolysin**: In transformed cell lines, double-stranded RNA viruses called reoviruses proliferate more easily than in normal cells (Vidal L *et al*. 2006, Hashiro G *et al*. 1977, Dumcan MR *et al*. 1978). Theoretically, the oncolytic	Head and neck cancer	A phase III is completed. It received an orphan drug designation from FDA.

		properties of reovirus depend on active Ras signalling (Norman KL *et al*. 2004; Bos JL *et al*. 2016). The only medicinal wild-type Reovirus currently being developed for clinical use is Reolysin. Of the many reovirus serotypes, the T3D strain has been studied the most as an anticancer agent (Heinhuis KM *et al*. 2019).		

Combining oncolytic virues with cancer treatments

In oncology, using viruses to treat cancer is an odd concept. When used in conjunction with traditional medicines, the effectiveness of treatment can be maximised. This combination can assist break through typical treatment resistance and improve the anti-tumor immune response of drug delivery. The combined force will cause the tumour to respond more strongly, extending its survival. More knowledge makes it easier to identify the best combinations of these treatments and the patient group that benefits the most, which leads to more efficient and customised cancer care.

The combination of OVs with chemotherapy

Cytotoxic chemicals are a key component of chemotherapy, a common cancer treatment that targets and kills cancer cells to reduce tumour size and possibly eradicate the disease. By preventing DNA replication and upsetting the cell cycle, these chemotherapy medications mainly target proliferating cells. Chemotherapy can damage certain benign growing cells, although it works best against cancer cells.Almost every chemotherapeutic medication has an adverse effect on non-cancerous cells that divide. (Wennier ST *et al*. 2012, Heinhuis KM *et al*. 2019). Additionally, a lot of tumours become resistant to chemotherapy medications, which frequently results in treatment failure. One benefit of oncolytic virotherapy is that it minimises harm to healthy tissues by using viruses to specifically target cancer cells. Curiously, studies indicate that chemotherapy may reduce the quantity of viral particles required to cure the tumour. According to recent research, the combination of oncolytic viruses and chemotherapeutic medications greatly increases efficacy when compared to the use of either treatment alone. By increasing the sensitivity of cancer cells, this combined method raises the treatment's overall efficacy. In 2005, China authorised the use of Recombinant Adenovirus-based Oncorine (rhAd5), also referred to as H101 (Liang M *et al*. 2018), in conjunction with chemotherapy to treat nasopharyngeal cancer.

The combination of Oncolytic Virus with radiotherapy

By exposing tumour cells to ionising radiation, which either directly breaks their DNA or produces oxygen radicals that interfere with biological functions, radiotherapy targets tumour cells. Combining this strategy with oncolytic viruses has two effects. By blocking the repair of DNA damage through the sequestration of DNA repair proteins, OVs can effectively act as chemotherapeutic sensitisers and increase the effectiveness of radiation therapy.Tissue-associated antigens (TAA) and Damage Associated Pattern Receptors (DAMPs) are released when radiation therapy causes tumour cell

death through necrosis or apoptosis. This encourages OV replication and spread to the malignant cells. Furthermore, radiation can boost viral replication, promote viral expression, and improve viral uptake. Research has shown that OVs and radiation therapy together yield better therapeutic results than either treatment alone. A promising method for adding immunotherapy to the treatment of patients with newly discovered diffuse intrinsic pontine glioma (DIPG) has recently surfaced: the combination of oAd DNX-2401 and radiotherapy (Nikolova E *et al*. 2021, Markert JM *et al*.2014, Dorta-Estremera S *et al*.2018, Liu XY *et al*.2005).

The combination of Oncolytic Virus with Immune Checkpoint Inhibitors (ICI) therapies

Since tumour cells and some immune cells frequently express PD-L1, which enables tumour cells to evade the immune system, immune checkpoint inhibitors can unleash an immune system onslaught on cancer, which is typically inhibited by tumour cells. By inhibiting the interaction between PD-1 and PD-L1, PD-1/PD-L1 inhibitors stop tumour cells from evading the immune system via this pathway. This inhibition improves the immune system's ability to recognise and eliminate cancer cells by restoring the immune cells' ability to target tumir cells. Pembrolizumab (Keytruda), Nivolumab (Opdivo), Atezolizumab (Tecentriq), Avelumab (Bavencio), and Durvalumab (Imfinzi) are a few PD-1/PD-L1 inhibitors that are used as medications to treat advanced melanoma, non-small cell lung cancer, renal cell carcinoma, bladder cancer, and Hodgkin lymphoma, among other tumour types.(Luo C. and others, 2022).

According to a study, the oncolytic vaccinia virus stimulates PD-L1 expression in tumour cells and immune cells in the tumour microenvironment, as well as recruiting effector T cells. In addition to lowering the number of PD-L1-positive cells, oncolytic virus therapy and anti-PD-1/PD-L1 therapy encourage effector CD8+ and CD4+T cells to infiltrate tumour tissues and raise IFN-γ, ICOS, granzyme B, and perforin expression, which kills tumour cells (Liu Z *et al*., 2017). In a phase 1b clinical trial conducted in 2017, Ribas *et al*. revealed that oncolytic virotherapy using T-VEC enhanced the number of CD8 T cells and the expression of the PD-L1 protein, thereby improving the effectiveness of pembrolizumab treatment. Furthermore, preclinical and clinical evidence has demonstrated that OVs may also be used as neoadjuvant agents to sensitize and improve therapeutic effects of subsequent tumor resection and ICI therapy (Jiang Y *et al*.2019).

The combination of Oncolytic Virus with CAR-T cell therapies

The process of designing, modifying, and growing T cells in vitro so that they can recognise tumor-specific antigens using the CAR structure produced on their surface is known as chimeric antigen receptor (CAR) T cell therapy. Because of this adaptability, CAR-T cells can enter the tumour microenvironment (TME) and target tumour cells that are expressing the relevant antigens. Acute lymphoblastic leukaemia (ALL) and chronic lymphocytic leukaemia (CLL), two haematological cancers that were previously incurable, now have a new treatment option thanks to CAR-T cell therapy. For the treatment of refractory B-cell lymphoma, the FDA authorised CD19-targeting CAR-T cell products in 2017. However, the therapy has only produced limited and transient responses in individuals with solid tumours. This is probably because "cold" tumours have less effector function and insufficient CAR-T cell penetration into the TME. Therefore, to increase the effectiveness of CAR-T cell therapy in both solid tumours and blood malignancies, novel combination techniques are desperately needed. As adjunctive medicines for CAR-T therapy, oncolytic viruses (OVs) have a lot of promise. OVs are well-positioned to supplement the therapeutic benefits of CAR-T cells due to their ability to cause viral infections and the immunological checkpoint blockage (ICB) of tumour cells. In preclinical research, a number of OV types have been designed to carry immune checkpoint-targeting compounds, T-cell-attracting chemokines, or immunostimulatory cytokines. Within solid tumours, these alterations may facilitate CAR-T cell migration, activation, and proliferation. An example of this would be the use of mesothelin-directed CAR-T cell therapy in immunodeficient mice with human pancreatic ductal adenocarcinoma (PDA) xenografts in conjunction with an oncolytic adenovirus expressing TNF-α and IL-2 (Ad-mTNF-a-mIL2). By stimulating M1 macrophage polarisation and promoting dendritic cell maturation, our approach improved the immunological milieu while enhancing the invasion of CAR-T cells and host T cells into immunosuppressive PDA tumours. Similarly, in a subcutaneous tumor-bearing mice model, Moon *et al*. used a modified oncolytic vaccinia virus (VV.CXCL11) designed to express CXCL11, a CXCR3 ligand, to promote T cell trafficking into tumours. Furthermore, it has been demonstrated that immune co-stimulatory molecules such OX40L, 4-1BBL (CD137), GITRL, and CD40L increase the local activation and proliferation of effector immune cells within the TME. Therefore, there is a lot of promise in combining CAR-T cells with OVs designed to provide immunological co-stimulatory chemicals. Even though tailored OVs are good at delivering therapeutic compounds, OV-based therapies are hampered by issues such

systemic viral clearance, restricted tumour targeting, and decreased infectivity. Using CAR-T or other antigen-specific T cells as carriers to deliver OVs to the TME is an alternate strategy to deal with these problems. CAR-T cells can release and deposit viruses at different tumour sites before being neutralised by antiviral mechanisms since loading them with tiny doses of viruses has no effect on their expression or function of CAR receptors.

Limitations and newer strategies to improve treatment with OV therapy

Preclinical challenges

The assessment of OVs using the mouse models available today is impacted by a number of constraints. First off, many mammalian viruses intended for human usage have stringent cell tropism, which frequently prevents them from activating in mouse models. The human immune system may potentially eradicate OVs before they reach the locations of tumours. Neutralising antiviral antibodies produced by the therapy or pre-existing antibodies can stop tumour cell lysis and OV multiplication within tumours during OV treatment. The quick removal of OVs can also be facilitated by complement activation, antiviral cytokines, and macrophages. While vaccinia virus generally infects most cell lines, including mouse and human-derived cancer lines, many mouse cells are resistant to HSV1, with notable exceptions like A20 lymphoma and D4M melanoma cell lines (Bommareddy PK *et al*. 2018). Although some degree of infection and replication may occur, the infection efficiency of human adenoviruses in murine cells is much lower than in human cells. This is primarily because murine cells lack the receptors required for adenovirus attachment (Blair GE *et al*. 1989, Ganly I *et al*. 2000). Due to the use of replication-deficient vaccine vectors in the majority of viral immunology research, little is known about how the immune system reacts to replicating adenoviruses (McElrath MJ *et al*. 2008). The Syrian hamster model has been useful for oncolytic adenovirus research because it is immunocompetent and tolerant for replication, which supports human adenovirus replication (Thomas MA *et al*. 2006). Humanised mouse models, immunocompetent hosts with robust adaptive immunity, or immunocompromised mice strains may be necessary in many situations; nevertheless, these models might not include the specific cell types or molecules essential to investigate OV interactions with the human immune system. Furthermore, subcutaneous tumour models do not effectively represent the tumour microenvironment (TME) or the features of numerous metastatic lesions. Although orthotopic mouse models can offer a comparable milieu, it is still difficult to get the virus to the tumour site. Although they frequently have lengthy latency periods, patient-derived xenograft (PDX)

mouse models provide a more accurate genetic depiction. Patient-derived organoid (PDO) models are helpful tools for OV testing because they maintain the heterogeneity, hypoxic circumstances, and many differentiation phases observed in actual tumours. PDO sensitivity to OVs may be instructive, as evidenced by the reaction of oncolytic adenovirus in pancreatic and renal cell cancer PDOs.

Balance between antiviral and antitumor immune responses

The equilibrium between antiviral and antitumor immune reactions to achieve optimal tumor regression remains a significant obstacles for OVs (Riera Romo M *et al*.2021, Krysko DV *et al*.2012) Especially for oncolytic virus derived from HSVs or adenoviruses which are endemic , pre-existing cross-reactive antibodies could hinder effective viral proliferation to individual previously exposed to similar viral family.(Wakimoto H *et al*.2002, Ikeda K *et al*.2000) .Additionally , advanced-stage of malignancies often require repeated OV injections, which may lead to the devolopment of neutralizing antibodies . Various strategies are being explored to reduce antiviral immunity. Protective coatings with chemical polymers,liposomes, or cell-derived nanovesicles are under investigation to sheild oncolytic virus from immune components. (Xia M *et al*.2019, Lv P *et al*.2019, Nosaki K*et al*.2016) though these protectively coated OVs are very tough to store and have higher production expenses. Another approach involves minimizing early viral clearance using ex vivo with oncolytic viruses. Certain immune cells, such as mesenchymal stem cells (MSCs), macrophages, dendritic cells, T (T) cells that are capable of migrating into tumors serve as a potential cellular carriers for oncolytic virus.(Mader EK *et al*.2009, Muthana M *et al*.2009). These carriers are helps to transporting OVs to the brain, particularly when treating CNS tumors. As an example, the tumor-tropic mesenchymal stem cells carrying adenovirus-based OV ICOVIR17 wew shown to improve survival in Glioblastoma models and selectively accumulate in brain lesions. (Martinez-Quintanilla J *et al*.2011)

Clinical challenges

Currently, the most promising delivery route for oncolytic viruses is intramoral injection. It can decrease both injected and noninjected tumour locations, according to preclinical and clinical experiments, suggesting distant therapeutic effects. The main obstacle to the clinical use of OVs is the tension between the requirement for intratumoral injection and the clinical requirement for intravenous distribution. When tumours are physically inaccessible, intravenous distribution eliminates the requirement for

localisation technicians and allows for extensive OV infection to all lesions. While there are certain benefits to intravenous oncolytic virus delivery, there are also a number of disadvantages to take into account. First, the host body's immune system may eradicate virus particles, lessening the effect even more. Furthermore, the optimal dosages are still unknown once the virus has been diluted in the peripheral circulation, making it difficult to determine the bioavailable concentration at the tumour site. Thus, new strategies to prevent neutralisation need to be created. To shield oncolytic viruses from neutralising antibodies, a number of strategies have been studied, such as retargeting, the use of cell carriers, coating with polymers, and encapsulation in liposomes (Menotti L *et al*.2018, Yamamoto Y *et al*.2017, Berkeley RA *et al*.2018, Kim J *et al*.2015, Garofalo M *et al*.2021, Na Y *et al*.2019, Wang Y *et al*.2019, Duff MF *et al*. 1968). The intravenous delivery of OVs is being studied in a few early-stage clinical trials. A study on enadenotucirev, a chimeric oncolytic adenovirus, demonstrated that viral particles were reliably identified on resected tumors following intravenous administration prior to surgery (Garcia-Carbonero R *et al*.2017)

Safety concerns

OVs are highly replicating viruses and warrant caution due to the potential for unintentional spread from patients to others or the environment during clinical use. Standards and procedure for the storage management and application of OVs as well as meassure for a overdosing and proper disinfection of affected areas are essential, with multiple protocols now accessible .(Hom V *et al*.2019, Gutzmer R *et al*.2018, Harrington KJ *et al*.2017) So far, there have been no documented cases of transmission to contacts or exposure incidents. One study reveals that 8.4% of household exposures with T-VEC reported cold sores, who were not confirmed to have infections and had clinically mild symptoms Concerns also exist regarding viral shedding, especially in immunosuppressed patients, and the potential of OVs containing recombinant DNA elements to recombine with naturally occurring wild-type viruses. (Macedo N *et al*.2020)

Conclusion

At this stage, it wouldn't be unjust to say that oncolytic viral therapy is a tried-and-true method of treating cancer. The effectiveness of oncolytic virus therapy is anticipated to increase further when paired with immunotherapy as the formation of specific antitumor immunity during oncolytic activities is a typical feature that plays a significant role in showing antitumor effects. More effective therapeutic approaches are needed to establish long-term tumour

control in patients, notwithstanding the promising preclinical and clinical findings for oncolytic adenovirus. Therefore, it seems sense that a variety of techniques are being employed to increase the oncolytic virus's potency. Better therapeutic options should soon be available as a result of the scientific and medical societies' decades-long efforts. A greater understanding of individual patient tumor immune state is anticipated to develop, allowing us treat advance cancer patients more effectively. It indicates that oncolytic virus will be used in future multimodality technique.

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Chapter - 4
Bioremediation Technologies for Petrochemical Effluents: A Comprehensive Review

Authors

Tania Deb
Ph.D. Student Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Pritha Pal
Assistant Professor, Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Sibashish Baksi
Assistant Professor, Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Chapter - 4

Bioremediation Technologies for Petrochemical Effluents: A Comprehensive Review

Tania Deb, Pritha Pal and Sibashish Baksi

Abstract

Petrochemical wastewater is highly contaminated with hazardous pollutants such as hydrocarbons, phenols, heavy metals, and volatile organic compounds, posing serious environmental and health risks. It exhibits high chemical oxygen demand (COD) and biological oxygen demand (BOD) due to toxic compounds like benzene and toluene, making conventional treatment methods inefficient and costly. In bioremediation process living organisms, primarily microorganisms, are used to remove or neutralize contaminants from soil and water. This approach is particularly effective for treating hazardous waste, including petrochemical wastewater, by breaking down pollutants into less harmful substances. Recent research highlights the development of advanced bioremediation technologies, particularly the integration of anaerobic and aerobic processes in bioreactors to enhance degradation efficiency. Advanced Oxidation Processes (AOPs) combined with biological treatments show promise for improving pollutant removal. The role of microorganisms-bacteria, algae, and fungi-in these processes is crucial, though this literature review focuses on the bacterial bioremediation, necessitating further exploration of microbial interactions to optimize bioremediation strategies. Overall, there is a strong emphasis on creating sustainable solutions for industrial wastewater treatment.

Keywords: Petrochemical wastewater, hydrocarbons, heavy metals, chemical oxygen demand (COD), Biological oxygen demand (BOD), Bioremediation

Introduction

Petrochemical wastewater contains high levels of chemical oxygen demand (COD) and biological oxygen demand (BOD) due to compounds like benzene, toluene, ethylbenzene, xylene (BTEX), volatile organic compounds (VOCs), and polycyclic aromatic hydrocarbons (PAHs). The wastewater also includes dissolved minerals, phenols, and emulsified oil content, contributing to its complex composition. Additionally, petrochemical wastewater may

contain sulfur compounds, organic compounds (e.g., benzene, toluene), phenols, ammonia, cyanides, and suspended solids, which are harmful to both humans and ecosystems. Bioremediation technologies play a vital role in treating industrial effluents, utilizing various methods such as aerobic biological degradation and chemical reagents to address pollution concerns. Researchers have contributed significantly to the field, with studies focusing on substrate utilization, biofilm formation, and reactor performance optimization. The review of bioremediation technologies discusses the integration of anaerobic and aerobic treatment processes in bioreactors to enhance degradation efficacy for different industrial effluents. By exploring the use of microorganisms, plants, and advanced bioreactors, the literature aims to provide insights into optimizing effluent treatment processes and protecting the environment from the impacts of industrial activities.

Discussion

Petrochemical effluents can have significant and detrimental effects on the environment and public health. Here are some key impacts as follows:

Environmental Effects includes - Water Pollution: Contamination of rivers, lakes, and groundwater, affecting aquatic ecosystems and drinking water sources. High levels of toxic substances can lead to the death of aquatic life and disrupt food chains; Soil Contamination: Soil degradation and loss of fertility due to the accumulation of harmful chemicals. Alteration of soil microbial communities, which can affect plant growth and ecosystem health; Air Pollution: Volatile organic compounds (VOCs) can evaporate into the atmosphere, contributing to smog and respiratory issues. Emission of hazardous air pollutants can lead to serious health risks for nearby communities; Biodiversity Loss: Habitat destruction and pollution can lead to the decline or extinction of sensitive species. Disruption of ecosystems due to changes in water chemistry and availability of nutrients.

Health Effects includes-Toxicity: Exposure to toxic compounds like benzene, toluene, and heavy metals can cause acute and chronic health issues. Increased risk of cancers, respiratory diseases, and neurological disorders; Reproductive and Developmental Issues: Some petrochemical pollutants can disrupt endocrine systems, affecting reproductive health and fetal development; Community Impact: Communities near petrochemical facilities may face higher rates of illness and health disparities. Psychological impacts from environmental degradation and potential health risks.

Economic Effects includes -cost of Cleanup: Remediation efforts can be expensive and resource-intensive, burdening local and national economies;

Impact on Local Industries: Fishing, agriculture, and tourism can suffer due to contaminated resources, leading to economic losses.

The effects of petrochemical effluents are far-reaching, impacting ecosystems, human health, and local economies. Addressing these issues requires comprehensive management strategies and effective treatment solutions to mitigate pollution and protect both the environment and public health. Bioremediation is a process that uses living organisms, primarily microorganisms, to remove or neutralize contaminants from soil and water. This approach is particularly effective for treating hazardous waste, including petrochemical wastewater. Microbial bioremediation leverages the natural metabolic capabilities of microorganisms to degrade or transform these pollutants into less harmful substances.

1) Environmental impact of petrochemical industries and wastewater treatment

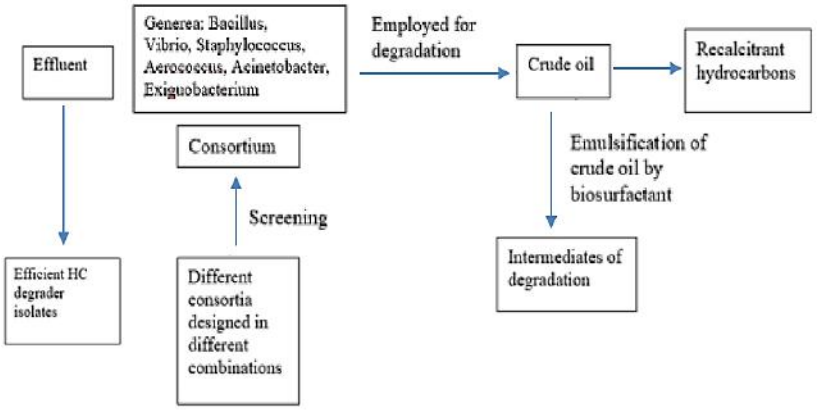
The rapid growth of petroleum and petrochemical industries has led to significant environmental pollution, primarily due to the hazardous wastewater generated. This wastewater is characterized by high levels of chemical oxygen demand (COD) and biological oxygen demand (BOD), largely due to the presence of compounds such as benzene, toluene, ethylbenzene, and xylene (BTEX). These effluents contain harmful substances such as crude oil products, polycyclic aromatic hydrocarbons, and heavy metals, which can severely impact aquatic ecosystems and human health (Biswas, T. *et al*, 2020) (Mohammed, N. *et al*, 2022).

Phenol as a Pollutant: Phenol is a significant environmental pollutant found in industrial wastewaters, particularly from petrochemical processes, oil refineries, and other related industries. It is recognized as a toxic substance and is listed as a priority pollutant by agencies like the World Health Organization (WHO) and the US Environmental Protection Agency (EPA). (Shahriari Moghadam. *et al*, 2016)

Toxic Compound	Structure	Toxicity
Benzene	Hexagonal ring with alternating single and double bonds	Carcinogenic, causes leukemia and other blood disorders
Toluene	Benzene ring with a methyl group attached	Neurotoxic, can cause respiratory problems, liver damage, and kidney damage
Ethylbenzene	Benzene ring with an ethyl group attached	Carcinogenic, can cause respiratory problems and liver damage
Xylene (o-, m-, p-)	Benzene ring with two methyl groups at different positions	Carcinogenic, can cause respiratory problems, liver damage, and kidney damage
Phenol	Benzene ring with a hydroxyl group attached	Corrosive, can cause skin burns and eye damage
Polycyclic Aromatic Hydrocarbons (PAHs)	Multiple fused benzene rings	Carcinogenic, can cause various types of cancer

2) Current treatment technology and bioremediation

The treatment of industrial wastewaters, particularly from petroleum refineries, is a significant challenge due to the complex chemical composition and high toxicity of the effluents. Various technologies have been developed to reduce hazardous pollutants, including flotation, coagulation, membrane separation, adsorption, and electrochemical oxidation. However, these methods often prove to be uneconomical and ineffective, as they can increase the final COD in the effluent due to the addition of chemicals, this has led to a growing interest in eco-friendly alternatives, particularly bioremediation, which utilizes microbial communities to degrade pollutants. This method is preferred due to its minimal disturbance to the environment and its reliance on naturally occurring organisms. Particularly aerobic processes, is preferred due to its cost-effectiveness and complete mineralization capabilities. (Biswas, T. *et al*, 2020) (Karray, F. *et al*, 2020) (Obukohwo *et al*.) (Nagda, A. *et al*, 2020) (Shahriari Moghadam. *et al*, 2016)



3) Community and diversity analysis

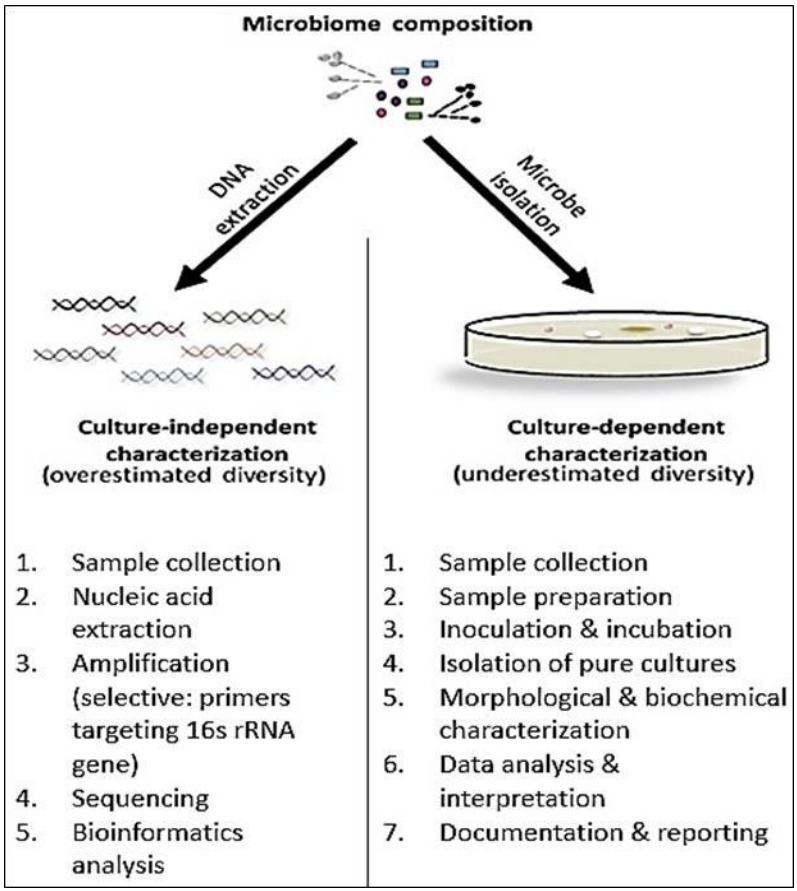
Culture-Dependent vs. Culture-Independent Methods: The literature indicates that many previous studies relied on culture-based methods for isolating and characterizing bacterial species. However, the paper notes the advantages of culture-independent techniques, such as PCR-based amplification of 16S rRNA genes, which have been successfully applied to analyse complex bacterial communities in various environments, including biological WWTPs. (Behnami, A. *et al*, 2018)

Understanding the microbial communities in wastewater treatment systems is crucial for optimizing performance. Traditional methods of characterizing these communities have limitations, as they often miss a large fraction of the microbial population. Recent advancements in genomics and next-generation sequencing (NGS) technologies have allowed for a more comprehensive analysis of microbial communities, revealing their structure, dynamics, and metabolic pathways involved in pollutant removal (Karray, F. *et al*, 2020).

The study involved isolating 31 indigenous bacterial strains from the wastewater of an effluent treatment plant. These strains were characterized using polyphasic taxonomy and 16S rDNA sequencing, revealing their potential for hydrocarbon degradation and enzyme production. The isolates formed a consortium that demonstrated significant BOD reduction in laboratory conditions. (Biswas, T. *et al*, 2020)

The study identified a total of 20 bacterial species using 16S rRNA sequence analysis, with some species being reported for the first time in a petrochemical WWTP. The Shannon diversity index was utilized to measure bacterial diversity, revealing a decrease in diversity from warm to cold seasons, indicating seasonal variations in bacterial communities. (Behnami, A. *et al*, 2018)

The study in question contributes to the growing body of literature on microbial biodiversity in activated sludge, particularly in the context of petrochemical wastewater. It identifies a total of 31 phyla, 65 classes, 146 orders, 167 families, and 185 genera within the bacterial community, with the domain Bacteria representing 94.9% of the total sequences. (Themis *et al*. 2020).



4) Findings of competent strains

The literature identifies key functional microorganisms involved in the degradation of hydrocarbons in petrochemical wastewater, such as Anaerolineaceae, Pseudomonas, and Sphingobium. These genera are essential for optimizing biological treatment processes. (Wang, Q. *et al*, 2020)

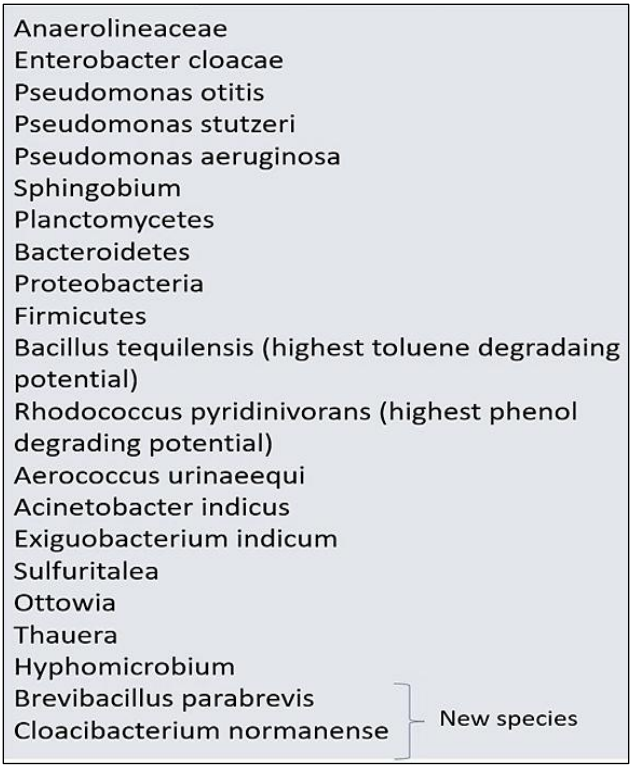
Research has identified key bacterial phyla, such as Proteobacteria and Firmicutes, as predominant players in hydrocarbon degradation. Over seventy -nine genera of bacteria have been documented as hydrocarbonoclastic, with many belonging to the subphyla of Proteobacteria and Firmicutes. Specific species, particularly from the genus Bacillus, have been recognized for their ability to degrade hydrocarbons effectively. (Biswas, T. *et al*, 2020)

Several bacterial species can degrade phenol, with Rhodococcus sp. being particularly noted for its ability to break down complex synthetic compounds.

This bacterium is capable of degrading substances that are otherwise resistant to degradation by other organisms. (Shahriari Moghadam. *et al*, 2016).

The research found that the dominant phylum in the activated sludge samples was Proteobacteria, which constituted 63% of the total isolates. This finding aligns with previous studies that have identified similar dominant groups in activated sludge from various wastewater treatment systems. (Themis *et al*. 2020).

The study identifies six exogenous bacterial strains known for their biodegradation capabilities. These include Pseudomonas stutzeri, Pseudomonas aeruginosa, Enterobacter cloacae, and others. The selection was based on their proven ability to degrade organic contaminants effectively. (Mohammed, N. *et al*, 2022).



Findings of competent strains

5) Factors influencing bioremediation

Several case studies show that highlight the variations in microbial communities across different treatment plants. These studies provide insights

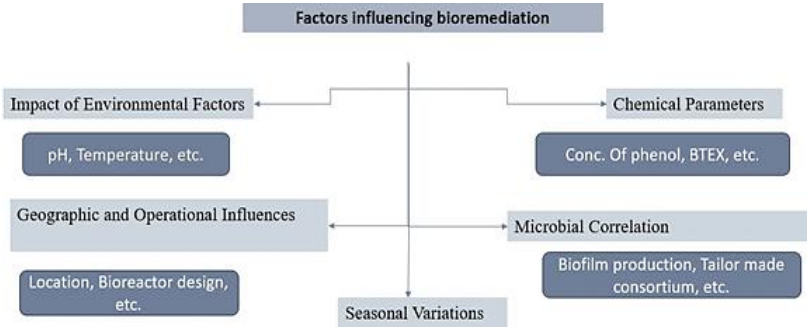
into how specific microbial populations respond to varying operational conditions and pollutant loads. (Kim, E. *et al*, 2020).

Impact of environmental factors: The literature indicates that environmental factors such as temperature, pH, and chemical composition of wastewater significantly influence microbial community structure and bioremediation process. The paper emphasizes the need to consider these factors when assessing microbial dynamics in treatment plants. (Kim, E. *et al*, 2020) For instance, while temperature is generally a critical factor, the study found that at the average temperature of 30°C, it did not significantly affect phenol degradation by an isolated strain. (Shahriari Moghadam. *et al*, 2016).

Seasonal variations: The research also emphasizes the importance of seasonal variations in microbial composition, noting that different seasons yield distinct microbial communities. For instance, the phylum Armatimonadota was notably dominant in the spring sample, correlating with higher chemical oxygen demand (COD) and total suspended solids (TSS). (Themis *et al*. 2020).

Chemical parameters and microbial correlation: The literature indicates a strong correlation between microbial communities and physicochemical parameters such as ammoniacal nitrogen (AN), total organic carbon (TOC), and dissolved oxygen (DO). Canonical Correspondence Analysis (CCA) was employed to explore these relationships, revealing that certain microbial taxa are associated with specific chemical conditions. The study reported a positive correlation between phenol and cadmium levels during the bioremediation process. (Themis *et al*. 2020) (Obukohwo, K. *et al*, 2020).

Geographic and Operational influences: Prior studies indicated that geographic location, wastewater characteristics, and operational parameters significantly affect microbial community composition. Notably, 70% of the variance in microbial communities was attributed to these factors in sewage treatment systems, highlighting the need for more research in industrial wastewater contexts. (Chen, Q. *et al*, 2017).



6) Bioreactor strategies

Membrane bioreactor or MBR systems represent a significant advancement in wastewater treatment, offering advantages over traditional activated sludge processes, such as higher sludge age and better effluent quality. Studies indicate that MBRs can effectively treat petroleum refinery wastewater, although most research has focused on treatment performance rather than the microbial communities involved.

Quantitative assessments: The study employs quantitative real-time PCR to assess the abundance of bacterial communities in Continuous Stirred Tank Reactors (CSTR) and MBR systems. Results show that bacterial populations dominate in both systems, with a significantly higher abundance in the MBR compared to the CSTR.

Performance metrics: The performance of the CSTR and MBR systems in treating petroleum refinery wastewater is evaluated based on various parameters, including Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD). The CSTR showed lower removal efficiencies compared to the MBR, which achieved higher biomass concentrations and better pollutant removal rates at lower hydraulic retention times. (Karray, F. *et al*, 2020)

Hybrid bioreactors: The survey indicates that hybrid bioreactors, which combine different treatment processes, show great promise for future applications. These systems can enhance pollutant removal efficiency and are suggested to be a focus for further research and industrial adaptation.

Coupled anaerobic-aerobic methods: The literature suggests that coupled anaerobic-aerobic methods are advantageous for treating high-strength industrial wastewater. This approach benefits from the higher removal efficiency of volatile suspended solids and chemical oxygen demand (COD) by aerobic processes, alongside biogas production from anaerobic

digestion. (Nagda, A. *et al*, 2020) (Sakdapetsiri, C. *et al*, 2021) (Mohammed, N. *et al*, 2022)

3D surface graph from RSM technology to determine optimum percentage of BOD reduction (Biswas, T. *et al*, 2020)

Conclusion

Advancing Environmental Protection: The review emphasizes the significance of bioremediation technologies in treating industrial effluents, highlighting their role in environmental protection and pollution mitigation.

Optimizing Treatment Processes: By exploring integrated anaerobic-aerobic methods and Advanced Oxidation Processes (AOPs) in bioreactors, the literature aims to optimize treatment processes for higher pollutant removal efficiency and sustainable remediation solutions.

Enhancing Bioremediation Strategies: Understanding microbial interactions and the application of hybrid bioreactors hold promise for enhancing bioremediation strategies, offering synergistic advantages and improved treatment capabilities.

Addressing Industry-Specific Challenges: The research underscores the importance of developing efficient and cost-effective bioremediation technologies tailored to different industrial sectors to address specific challenges and ensure environmental sustainability.

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Chapter - 5
Ubliquitous & Noxious Mercury: Effect on Reproductive system of an Organisms: A Review of Evidence

Authors

Souradeep Banerjee
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Mayisha Sultana
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Tanya Das
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Tuhin Mukherjee
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Sovan Patra
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Priyankar Pal
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Chapter - 5

Ubliquitous & Noxious Mercury: Effect on Reproductive system of an Organisms: A Review of Evidence

Souradeep Banerjee, Mayisha Sultana, Tanya Das, Tuhin Mukherjee, Sovan Patra, and Priyankar Pal

Abstract

A hazardous substantial metal that occurs innate form in the Environment is Mercury, which has a negative effect on the majority of organisms reproductive capabilities. Reproductive organs are vital for adaptation and proliferation of a species but this metal released into the air, water and soil by human activity. Mercury metabolized by bacteria into methylmercury are profoundly hazardous and posses severe toxicity risks in the ecosystem. In reproductive toxicology, A crucial role plays by mercury in disrupting reproductive mechanisms of both the genders such as affecting sperm levels, motility, viability, sperm generation, hormone disbalance, follicular deterioration, and delayed maturation of oocytes.The experimental research that used rats, mice, birds and rabbits to clearly shows that how mercury affects the structure and functionality of reproductive organs. Prior to its terrible effects on humans and animals, mercury was utilized in medicine. The most potent neurotoxin known today is mercury, which has several negative health consequences on both people and animals. Consequently, in order to minimize use and exposure, it becomes vital to educate people about the effects of mercury on reproduction about its incoming source to the environment and its treatment for Detoxification

Keywords: Heavy metal, methylmercury, reproductive toxicology, neurotoxin

Introduction

A fundamental process of life for generating new offspring and supporting the advancement and endurance of a species is reproduction. The reproductive system affects an organism's behaviour which further regulates the anatomical growth and physiological changes between males and females. Mercury was known to the ancient Chinese and Hindus before 2000 BC and

was found in tubes in Egyptian tombs dated from 1500 BC. Aristotle and Hippocrates (c. 350 B.C.) called it quicksilver and employed it in medicinal preparations. The acronym “Hg” comes from Pliny the Elder’s term “Hydrargyrum” (Hydra-liquid, or water; Gyrum-silver).Mercury is a widespread and persistent heavy metal with the potential to cause chemical contamination. It exists naturally in the environment in three forms:

Mercury was familiar to ancient Chinese and Hindu civilizations before 2000 BC and was discovered in vessels from Egyptian tombs dating to 1500 BC. Aristotle and Hippocrates (circa 350 BC) named it quicksilver and employed it in therapeutic mixtures. The acronym “Hg” comes from Pliny the Elder’s term “Hydrargyrum” (Hydra means liquid or water, whereas Gyrum means silver). Mercury is a widespread and persistent heavy metal with the potential to cause chemical contamination. It naturally occurs in the environment in three forms:

1) HgO (metallic), a lustrous, aromatic-free liquid that is silver-white at normal temperature, or mercury vapors that evaporate at higher temperatures.
2) Hg2++ (mercurous), is found mostly as white powders or crystals when coupled with oxygen, sulfur, or chlorine as inorganic mercury salts.
3) Hg++ (mercuric), refers to an organic mercury compounds that have been mixed with carbon.

In the atmosphere vapor of an elemental mercury, can linger for up to a year, makes up the majority of the mercury in the surroundings. Thereby, mercury vapor can travel thousands of miles from potential emission sites and can be extensively distributed. Most of the mercury foun in plants, animals, soil, sediments, and water exists as inorganic and organic mercury salts. Mercury was used as medicine for a long time, but the first indication of organo mercurial poisoning was noted by Ramazzini in 1713.Although there are theoretically many different organic mercury molecules (dimethyl mercury, phenyl mercury, ethylmercury, and methylmercury).Whereas, methylmercury is the most prevalent one in the environment. The build-up procedure of methylmercury in fish and seafood has been linked to the majority of reported health hazards to humans. Infertility affects 15% of couples of reproductive age, making it a significant public health challenges. Exposure of a hazardous materials in the workplace and environment causes a variety of changes to the biological system. The list of harmful mechanisms includes ion mimicry, oxidative stress, disruption of cell signaling pathways,

epigenetic regulation, damage to the blood-testis barrier, apoptosis, changes in gene expression, inflammation, and disruption of an endocrine system. Diverse levels of environmental contamination and rearrangement of mercury in the food chain are the outcomes of industrial development and agricultural practices. There are some elements which is vital to biological systems and others that have been characterized as extremely hazardous.

In Males, reproductive problems such as subfertility and sterility, are intriguing when considering their environmental implications. Recent years have seen an increase in awareness of negative trends in male reproductive health link with an environmental factors. Over the past 50 years, reports have indicated in certain places that decreasing in spermatozoa counts and also increasing in reproductive disorders. Due to the relatively small time span it is hypothesized that environmental toxic metals like Mercury influences and have a more significant impact than hereditary elements. Due to the changes, male reproductive system functions can influence reproductive health and act as a highly sensitive indicator of environmental dangers. Physical and chemical exposure have been the most often documented risk factors for male reproductive function. The hazardous compounds effects at low exposure levels on the structure and function of Male reproductive system which are well-demonstrated through dosages and exposures. The majority of populations are exposed to hazardous compounds like mercury, which is frequently linked to impaired reproductive function.

In Females, mercury has been demonstrated to impede the anterior pituitary's ability to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). This may results uterus to tilt the levels of progesterone and estrogen to tip, which may result in painful or irregular periods, an early onset of menopause, and various ovarian malfunctions. Numerous menstrual issues, including discomfort, irregular flow, and menstrual periods that are either shorter or longer than usual, have been linked to mercury exposure.

Sources and Distribution of Methylmercury

Two primary pathways allow mercury to enter into the environment are natural and man-made. The biosphere can receive mercury in various forms, such as mercuric halide (25%), monomethyl mercury (21%), vapour of mercury (4%), dimethyl mercury (1%), and particulate matters (4%).While there are currently no known substantial direct anthropogenic sources of methylmercury in nature, whereas indirect anthropogenic emissions do contribute to the natural methylmercury levels due to the transformation of other forms of mercury. A chronic bio-accumulative toxin, methylmercury has

been connected to a number of health issues in both humans and wildlife. The gastrointestinal tract absorbs the substance quickly. In both humans and animals, this type of mercury spread across the body and swiftly passes across the blood-brain and placental barriers. It transit into tissues that seems to be enhanced by the creation of a methylmercury-cysteine complex, which is transported into cells via neutral form of an amino acid carrier protein that is extensively distributed and shares structural similarities with methionine. In rats, methylmercury is gradually demethylated to generate mercuric mercury, making methylmercury in the body which is relatively stable. In humans, it has a rather lengthy biological half-life vary between 44 and 80 days. When administered intravenously, mercury compound is typically found in the kidney, liver, lungs, and right side of the heart whereas it is rarely seen in the spleen, extremities, or spinal column. Urine, breast milk, and excrement are the three ways excretion happens. Few studies have been done on absorption after inhalation exposure where Methylmercury exhibits a strong reactivity towards sulfhydryl groups, which encompasses reduced glutathione (GSH). Several rat tissues have been found to have a methylmercury GSH complex. Mercury vapor emitted by amalgam restorations in pregnant rats was studied. On that basis, the results showed that the mercury reached the placenta, liver, brain, and kidneys but not the lung. Positive associations were seen between the content of mercury and the surface areas of maternal amalgams, but not between the Mercury accumulation in the fetal brain, liver, or kidneys are observed. The organic form of mercury is the main type that crosses the placenta and is easily taken up into blood of fetal. Organic form of mercury levels the placenta are on par with or higher than those in mother's blood. Methylmercury hydroxide mostly affects early-elongated spermatids, premeiotic spermatocytes, and spermatogonial cells.

The Process of Biotransformation and Bio-magnification

The atmospheric residence time of mercury vapor is well-established for up to three years, while in soluble forms the residence time is only for few weeks. The initial phase of aquatic bioaccumulation involves the transition of mercury from its inorganic to methylation forms. This can happen by microbial action or non-enzymatic means. When methylmercury reaches predatory species food chains, bio-magnification takes place. The ultimate mercury sinks whereas the sediments found in lakes, seas, and the crust. Microbes can convert inorganic mercury into the poisonous chemical methylmercury, which easily builds up in water. Mercury undergoes both organic (demethylation) and inorganic (methylation) forms as part of kinetics and biotransformation, depending on its initial chemical and physical state.

Mercury compounds undergo biotransformation in large part due to the metabolic diversity of microbes. There are three possible valence states for inorganic mercury: 0, +1, and +2. Aerobic bacteria breaks down mercuric sulfide which further converts sulfite and sulfate and thereby solubilizing Hg2+ from sulfide. Several bacterial species include the NADPH-dependent flavoenzyme mercuric reductase, which solubilizes mercury and then reduces it to the volatile, while low-toxicity forms which known as mercuric oxide (HgO). The primary producers of methylmercury (CH3Hg) in anoxic waters and sediments which harbor sulfate-reducing bacteria (SRB) though little information about the biochemical mechanism of methylmercury synthesis has been reported. Desulfovibrio desulfuricans & One sulfate reducing bacteria (SRB) strain has been shown to exhibit mercury methylation in part due to the acetyl-coenzyme A (CoA) route, a reversible process in carbon metabolism that turns acetate into carbon dioxide. However, this finding was not later verified in other species.

Another potential location for mercury methylation has been suggested to be the methionine biosynthesis pathway. Neurospora crassa, an aerobic fungus, isn't thought to be a significant natural source of methylmercury, but it has been implicated in the biological methylation of mercury via methionine synthase, which further adds methyl group to homocysteine. In the year of 1969, Scientists from Sweden and Japan found that fish acquire methylmercury. In summary, little fish takes up the methylmercury from the water. Fishes near the apex of the aquatic food chain accumulates highest quantities of methylmercury as they are eaten by larger fish. For worldwide research mercury is a major topic because of its long-range atmospheric transport, conversion into more dangerous substances, potential of some forms engage in photochemical reactions, which is a phenomenon called as biomagnification within the aquatic food chain.

Effects of mercury on reproductive toxicity

During pregnancy, exposure to even low quantities of hazardous metals throughout the fetal development stage can result in irreversible developmental problems due to their capacity to accumulate. Women who are childbearing age group are mostly at the risk because they are the most vulnerable to methylmercury exposure throughout the fetal developmental stage.

Due to the extreme toxicity of methylmercury, it can cause neurodevelopmental delays or even foetal death. The brains development is particularly vulnerable to long-term harm caused by environmental toxins.

There are several anomalies which tells that offspring of pregnant mother who were subjected to relatively elevated peak of methylmercury may display delayed walking and talking, lower neurological test scores and learning deficiencies.

Methylmercury easily enters into the fetal circulatory system through pregnant women who eat excessive amounts of seafood. Researchers showed that mothers' consumption of fish had a direct impact on the amount of blood in their offspring that contained mercury in a two-year trial including 1,057 infants in Hong Kong. In 24.7% of the newborns, the mercury concentration in their cord blood above the upper permissible limit is 61 nmol/L, while 3% of the babies had amounts over 100 nmol/L. The degree of safety of maternal exposure during pregnancy is still up for debate. Methylmercury can cause intellectual impairment, stillbirth, cerebral palsy, delayed speech and issues in controlling chewing, swallowing and salivation. It just takes four days for methylmercury to be easily taken up by the bloodstream and circulated to all body tissues, involving the brain of an unborn baby. The cause for the fetus's increased susceptibility to methylmercury and its distinct impact of neural pathology as compared to adults, has not yet been determined in studies.

Recent research has shown that there is a substantial risk (>30%) for a neurocognitive disease in the child, if the mother's peak hair mercury level is more than 70μg/g. If one baby born to a mother who consumed too much methylmercury, display a number of neurological issues as well as delayed development.

Mercury can impact on fetus's reproductive system and impairing the growth of reproductive system of a male which effects in low testosterone, fewer sperm, and damaged sperm cells. Reduced fertility is the major outcome of mercury exposure on the reproductive systems of both males & females. Mercury exposure may cause the metal to accumulate in testicular tissue, which would therefore hinder overall testicular function and particularly in spermatogenesis. Increased particulate fat in the sperm cells of rats treated with mercuric chloride suggested that the spermatozoa had deteriorated, which may have contributed to lower pregnancy rates amongst the exposed groups. In female rats, the concentration of toxic metals also affects the estrous cycle although not to the extent that causes fetal necrosis or early pregnancy termination. Methylmercury poisoning in mice affects sperm motility, count, and energy consumption. It also affects the epididymal architecture. The average number stating implantation sites where gradually decreased significantly and if it didn’t exist in females residing with 1661 treated males, indicating that all these factors leads to infertility failure.

During the 1950s in Minamata, Japan, the male-to-female ratio of stillborn fetuses gradually increased, which implies that male fetuses were more vulnerable to methylmercury contamination as compared to females. This results in decreased number of male progeny born during that period. The enzyme system known as 3-beta-hydroxy-delta-5-steroid dehydrogenase (3-beta-hsd) is essential for the synthesis of all types of steroids hormones. The inhibition of spermatogenesis by mercuric chloride may contribute to decreased pregnancy rates which inhibits the first step of sexsteroid-hormone production and testicular 3-beta-hsd activity. Progesterone accumulation is favorably linked by a considerable elevation in 3-beta-hsd activity in fish oocytes treated with mercury. At a mild level of intoxication, inorganic mercury binding to the plasma membrane of fish oocytes triggered steroidogenesis and translatable messenger RNA production. Progesterone production and 3-beta-hsd activity were first boosted by mercuric chloride treatment, the initial increase in these levels was reversed after depuration. High mercury exposure can have disastrous developmental consequences on the fetal nervous system since the metal easily crosses the placenta and possess serious harms on developing nervous system.

It is thought that methylmercury inhibits brain activities, such as neural cell migration and division, which are particularly important for growth and development. The effects of prenatal poisoning on newborns range from mild developmental delays to severe cerebral palsy. Background population exposures are linked to a wide range of issues, such as IQ, attention, memory, learning and social behavior deficits. Limited information about the outbreak regarding the low amounts of noxious methyl mercury exposure to the pregnancy on the newborn was obtained from studies of the Iraq mercury poisoning incident.

Bread was accidentally made using wheat seed that had received treatment with an alkyl mercury fungicide in the year of 1971-1972. 459 Iraqis lost their lives and another 6,500 were admitted to hospitals due to neurological problems. The majority of the mother had exposures which cause their hair levels to exceed the lowest levels linked to adult consequences. Based on a study of the data, there was an estimated 5% probability that fetuses whose mothers exhibited mercury level between 10 to 20 ppm mercury could be impacted. The U.S. FDA states that the average amount of methylmercury in the hair of those who have not been exposed is approximately 2 ppm.

Elimination of Mercury-Toxicity

Despite the extensive body of research on mercury and the general acceptance of its toxicity, mercury is still used in many forms, which has an adverse influence on an organism's ability to reproduce. Getting rid of the exposure source is the most crucial step in managing mercury poisoning. There is an immediate need for cleanup technology that can effectively and economically handle substantial quantities of soil, water, or sediment tainted by small concentrations of mercury. People have used plants for ages to treat everything from heart illness to croup. Using green plants, a technique known as phytoremediation helps clean up contaminated areas of pollutants. Phytoremidiation involves live green plants directly to break down, contain, or make different environmental pollutants harmless-such as heavy metals or persistent organic pollutants are resistant for remediation.

The utilization of the mercury-resistant bacterial genes which are merA and merB extracted from genetically engineered plants to perform phytoremediation of mercury or methylmercury. Highly poisonous ionic mercury, Hg(II), is changed significantly into safer and less volatile elemental mercury Hg(O), by the mercuric reductase gene product. Organomercurial lyase is an enzyme, which changes methylmercury into mercury (II), which is further encoded by the merB gene. Arabidopsis (a mustard plant) or Tobacco had their genes cloned, either alone or in combination. A different group of scientists altered the merA gene's genetic coding sequence to change a range of plant varieties, such as yellow poplar, tobacco, Arabidopsis and Brassica napus. In hydroponic studies, 80% of the ionic mercury was eliminated by merA-plants from the system within a week, whereas the elimination controls only 20% of it. Therefore, researcher found that plants that have undergone genetic engineering are able to detoxify and reduce remarkably higher rates of poisonous mercury.

Conclusion

Numerous carefully carried out research projects examining the consequences of methylmercury at concentrations beneath those in the Iraq incident have yielded conflicting results. The majority of research on mercury exposure at work focuses on elemental or inorganic mercury. Therefore, the effects are neurological and renal which are further noted at relatively lower exposure levels. Due to methodological flaws, some research examines that the effects of dental amalgam have been classified as inconclusive.

In summary, the subsequent conclusions can be drawn out from the collective research evidences:

The most accurate measure of negative consequences in people exposed to elemental and methyl mercury is neurotoxicity. In laboratory animals, elemental mercury is a developmental toxin. US EPA has not been provided the dose-response estimation for elemental mercury's developmental impacts.

In human development methyl mercury is detrimental. Based on information from human investigations, animal genetic toxicology research, and an analysis of methylmercury's pharmacokinetics, it is likely to be said that methylmercury is a mutagen of human germ cells. In experimental animals, toxic dosages of methylmercury and inorganic mercury causes tumors.

Based on available data and insights from ongoing research, the U.S. EPA projects the following results:

1) There will be a greater occurrence of neurotoxic consequences, if human populations are in direct contact with substantial quantities of elemental mercury.
2) Mothers who are in immediate contact with high concentrations of methyl mercury during pregnancy or after giving birth are more likely to experience neurotoxic consequences.
3) Reproductive effects or immune-mediated kidney damage are more common, if human populations are in direct contact with elevated concentrations of inorganic mercury.

We all knows that mercury is having harmful effects on the environment, animals, and people, so it goes without saying that we should consider a several ways to reduce its usage and emissions. This endeavor is not only the responsibility of one individual, but also the entire world. By using mercury-free substitutes and educating people on safe handling, secure storage and appropriate disposal techniques, we can reduce the amount of the toxic metal that is used. Healthcare professionals have the ability to enlighten the public on the risks associated with mercury exposure as well as the safe thresholds that the FDA has established.

The key to lower the exposure is to have a better understanding of the quality criteria of the items. Pregnant individuals, young children, and women in their childbearing age must avoid eating fish especially tuna, shark, swordfish, mackerel, and tilefish. Because this fishes contains excessive amount of mercury concentrations. In order to limit the impact on an organism's reproductive system, women who are pregnant or planning a family should stay away from employment exposure to mercury salts.

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Chapter - 6
Quantitative and Topical Analysis of Anthocyanins in Food

Authors

Sudipa Mukherjee
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Priyankar Pal
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Chapter - 6

Quantitative and Topical Analysis of Anthocyanins in Food

Sudipa Mukherjee and Priyankar Pal

Abstract

Anthocyanins are flavonoids being differential in colours and distinct health-promoting properties. These molecules are important in the field of nutritional science and food industries. This review article focuses on analytical methodologies of anthocyanin detection and quantification in food matrices. Quantitative and topical techniques will be compared in this article. Quantitative methods such as HPLC and MS offer precise quantification of anthocyanins requiring sample destruction, limiting their use in continuous quality control. Topical approaches such as NIR and hyperspectral imaging provide rapid, in situ analysis without causing any problem to sample integrity, ideal for on-site food quality analysis. The review points out improvements in chromatographic techniques, especially Ultra-high-performance Liquid Chromatography (UHPLC) coupled with modern detectors, which provide higher resolution in the analysis of anthocyanin. It also puts emphasis on the increasing role of topical techniques in the food industry for their efficiency and minimal sample preparation. The paper discusses both analytical realms' strengths and limitations, thus focusing on the current challenges and potential future development in the analysis of anthocyanin in food, giving light to future research directions.

What are Anthocyanins?

These are referred as a class of flavonoids being colourful, soluble pigments that are important for purposes other than just giving vegetables and fruits their respective colour. These compounds have high nutritional value in foods and are widely such different colourful berries, grapes, apples, plums, cabbage, and other grains and vegetables. Acknowledged for their possible health advantages, anthocyanins are being researched more and more for their potential to prevent and treat chronic illnesses like cancer, obesity, and cardiovascular disease. They are found in a wide range of food matrices, with berries, red wine, and colored grains like purple [1]. Anthocyanins can catalyse the activation of phase II enzymes through the antioxidant response element pathway against oxidative stress-induced apoptosis, highlighting their

potential to reduce oxidative stress and their chemo-preventive action [2]. These compounds reduce inflammation and improve glucose and lipid metabolism by inhibiting nuclear factor-kappaB activation and increasing PPAR-gene expression in metabolic syndrome subjects, having a significant role in metabolic health [3]. Anthocyanins also play a chemo preventive role in atherosclerosis via the activation of Nrf2 ARE as an indicator and modulator of redox, demonstrating their capacity to attenuate inflammation-associated pathogenesis by gene expression [4]. Additionally, anthocyanins' ability to modulate gut microbiota further contributes to their neuroprotective effects, with potential action as either complementary or standalone treatment options for various non-communicable diseases, especially neurodegenerative disorders [5]. Anthocyanin consumption has been associated with the maintenance or reduction of body weight in obese individuals, increased metabolism and energy balance [6].

The analysis of anthocyanins are required to screen their health promoting properties and bioavailability in food. Advanced techniques like nano emulsion and nanori prosome encapsulation are being explored to increase their stability and bioavailability in food related compounds [7, 8].

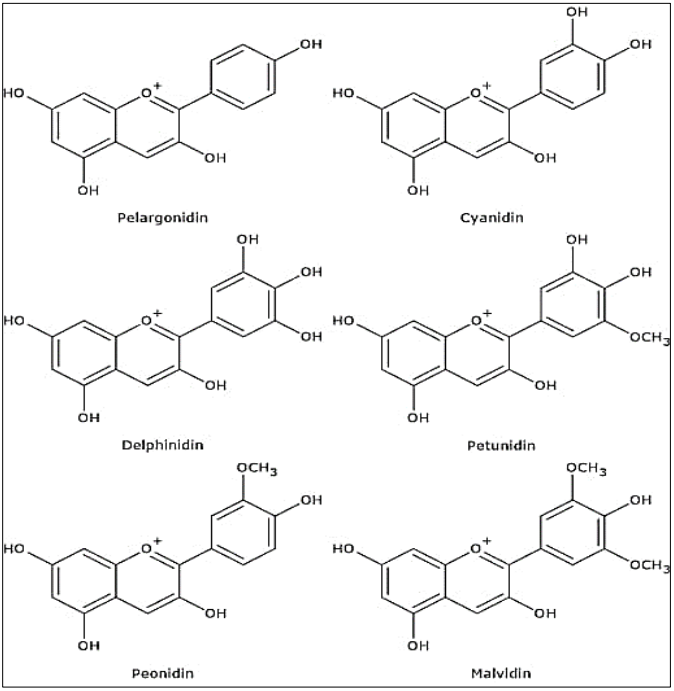

Fig 1: Chemical structure of different Anthocyanins such as pelargonidin, cyanidin, delphinidin, petunidin, peonidin and malvidin

This review article delves into the analysis of anthocyanins in food providing insights into differential techniques using chromatographic approaches with minimal amount of sample preparation and limitations of the technique for high throughput results.

Insights into the comparison between Quantitative and Topical Analysis of Anthocyanins

Anthocyanin analysis had been delved with the analytical technique of HPLC being combined with MS. The continuous requirement for confirming the identity of anthocyanins often requires complementary methods, such as nuclear magnetic resonance (NMR) spectroscopy or direct comparison with authorised regulatory standards for its efficiency [9]. The technique entails dissolving the food sample and allowing it to pass through a column containing a stationary phase, because anthocyanins interact differently with this phase in the presence of a flowing mobile phase, helping them to separate based on their different affinity [10]. When combined with modern detectors such as Diode Array Detection (DAD) and Mass Spectrometry (MS), UHPLC reveals fine data about anthocyanin profiles, to proficiently understand functions in food science and nutrition [11,12].. The results show that butterfly pea flowers can be used to extract anthocyanins, which can then be used as a safe food coloring [13]. The challenge is to improve these processes to guarantee both efficient extraction and anthocyanin purity. Maintaining the representativeness of the analysis depends on this careful balance [14]. The variety of anthocyanin structures found in food samples makes it difficult to standardize solvent compositions and create reliable extraction and analysis techniques. Although quantitative analysis techniques are essential for thoroughly characterizing the composition of anthocyanins, it is crucial to take into account their effects on the environment and the moral implications of using them [15]. Yet, innovations in chromatography, Mass Spectrometry, and sample preparation are projected to improve their precision, efficiency, and sustainability [16, 17].

In addition to maintaining the sample's integrity for possible future use, topical analysis techniques enable repeated sample measurements without changing the sample's physical or chemical state. Due to their simplicity, speed, and minimal sample preparation requirements, these methods are becoming more and more popular [18]. Spectroscopic methods such as Raman spectroscopy and NMR spectroscopy has shown greatly valuable results [19, 20]. These spectroscopic methods has been identified as one of the most adapted efficient protocol to be studied and performed for the analysis of anthocyanins [21].

Both topical and quantitative procedures have distinct functions in analysis, and the choice of either is based on the particular objectives, sample properties, and resources at hand. The most thorough understanding is frequently obtained by combining the two approaches (Figure 2). The advancement of digital imaging technology has significantly enhanced the potential of topical approaches, even though quantitative methods are essential for accurate compositional analysis and the creation of food standards [22]. The combined analytical techniques of this anthocyanin analysis helps in better understanding of it's content in food.

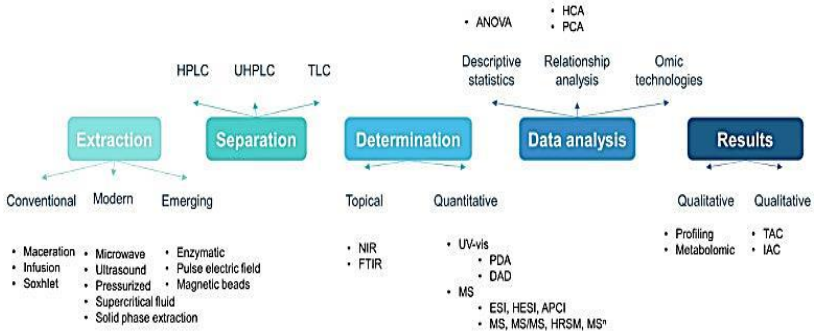

Fig 2: Schematic Diagram for Analysis of Anthocyanins in Food.

Quantitative analysis of anthocyanins in food

Evaluating a food's total antioxidant capacity is essential for quality control, nutritional profiling, and guiding consumer decisions. In reaction to environmental stimuli, it also helps researchers create functional foods and investigate health advantages. In this regard, determining the total anthocyanin content of food can be useful for rapid assessments of antioxidant capacity or sample comparisons. Analysing the different types of anthocyanins enriches antioxidant capacity estimations and provides a wealth of information, including an understanding of bioactivities and tailored dietary recommendations.

Because of their high sensitivity, selectivity, and resolution, liquid chromatographic (LC) techniques are ideal for examining complex mixtures, like those present in food and natural products [22, 23]. The basic idea behind LC's operation is the separation of analytes from a liquid sample by means of interactions between the stationary and mobile phases [24, 25, 26]. The sample is moved through the system by the mobile phase, which is usually a solvent or a blend of organic and aqueous solutions. Whether it is acidified or salt-enriched, its composition is determined by the methods or techniques used for separation. Solid or liquid, the stationary phase is placed into a column or

coated onto a support. Its composition varies depending on the kind of analyte: ion exchange for charged groups, size exclusion for size-based separations, normal phase for less polar, and reverse phase for polar. Although pressure and particle size requirements vary, High-performance Liquid Chromatography (HPLC) and Ultra-high-performance Liquid Chromatography (UHPLC) both use the same separation techniques based on affinity, size, and electrostatic interactions. UHPLC is a sophisticated type of HPLC that uses a smaller stationary phase and runs at higher pressures (10,000 to 15,000 psi).

High Performance Liquid Chromatography

In a South Brazilian vineyard, LiColombo *et al*. (2020) examined the yield and phenolic composition of the seedless table grape known as "BRS Vitoria" at different bunch densities [27].

The effects of terroir, viticultural practices, vinification techniques, and aging conditions on anthocyanins and volatile compounds in wines were examined by Tsiolkas *et al*. (2020) [28]. Yiannoudi and Maratheftiko, two native Cyprus varietals from the 2014, 2015, and 2016 vintages, were the subject of the study. Using HPLC-DAD-ESI/MS, anthocyanin profiling and determination were carried out for up to 38 anthocyanins. For the study, a Poroshell 120 C18 column (2.7 µm, 50 × 4.6 mm) from Phenomenex (Torrance, CA, USA) was used, along with water and acidified (5% FA) methanol as mobile phases. According to the study, wines might be easily distinguished by variety in the early stages, especially based on the anthocyanin makeup. But as samples aged, variations were impacted by winemaking practices over time, making it difficult to identify different types in these circumstances.

A Varian Pursuit C18 column (3µm, 150 × 2.0 mm) from Agilent and 0.1% FA in MeOH and water as mobile phases were used to analyze 16 anthocyanins using HPLC-DAD-ESI coupled to High-resolution Mass Spectrometry (HRMS) in positive ion mode [29]. In order to analyze the 90 grape samples from 2017 and 2018, organic solvents were used for extraction. Phloroglucinolysis, HPLC-UV, and MS detection were used to ascertain the proanthocyanidin profile in both seeds and skins. Using 0.1% FA and MeOH as mobile phases and an HPLC-UV Pinnacle II C18 column (4µm, 250 mm × 4.6 mm) from Restek (Centre County, PA, USA), more than 13 anthocyanins were identified [30].

Using particular parameters, Xue *et al*. (2021) optimized the ultrasound-assisted extraction (UAE) method for cranberry anthocyanins, obtaining the maximum yield (7.25 mg/g) [31]. The ZORBAX Eclipse XDB-C18 column

(5μm, 4.6 mm × 150 mm) from Agilent, together with 5% FA and 1% FA in ACN, were used in HPLC-UV-MS/MS to discover seven anthocyanins, including new ones like peonidin-3-(6-malon)-glucoside and pelargonidin-3-(6-malon)-glucoside. The efficacy of the UAE approach suggests that it has the potential to extract bioactive components from cranberries.

The effectiveness of infrared (IR)-assisted extraction was examined by Barba *et al*. (2022) in comparison to the conventional water bath method and under Response Surface Methodology-optimized conditions [32]. The study highlights the potential of carrot colorants as stable and natural substitutes for synthetic and other natural colorants, arguing that their greater stability is due to their abundance in acylated anthocyanins [33]. Mackon *et al*. (2023) profiled the changes in anthocyanin content and gene expression dynamics at three developmental stages (milky, doughy, and mature) of black rice by combining transcriptome analysis and HPLC-PDA [34]. Jia *et al*. (2024) used HPLC-Q-Orbitrap MS to characterize the anthocyanin content of Gynura bicolor. Five different types of anthocyanin compounds were found in the study, with delphinidin 3-O-neohesperidoside being the most common. An Ultimate AQ C18 column was used for chromatographic separation [35].

HPLC Method for Quantitative Analysis of Anthocyanins in Food

HPLC is a high scale broadly used technique for the quantitative analysis of anthocyanins in food for its precise ability to separate complex mixtures.

1) Sample preparation

Anthocyanins are first extracted from food samples using solvents such as acidified methanol, ethanol, or water.

The extract is filtered and concentrated, often using a vacuum rotary evaporator, to prepare it for HPLC analysis.

2) HPLC Setup

Column: A reverse-phase C18 column is typically used because it separates anthocyanins in accordance with their interaction with the stationary phase and polarity.

Mobile Phase: A gradient of aqueous formic acid (or acetic acid) and methanol or acetonitrile is used to optimize separation.

Detector: Anthocyanins are detected using a UV-Vis detector at wavelengths between 510 and 540 nm, where they show maximum absorbance.

3) Separation and Detection

Anthocyanins are separated based on their molecular size, polarity, and chemical structure.

Each anthocyanin appears as a distinct peak in the chromatogram, and their retention times help identify specific anthocyanin compounds.

4) Quantification

A standard, typically Cyanidin-3-glucoside, is used to create a calibration curve.

The concentration of anthocyanins in the food sample is quantified by comparing the derived chromatogram peak areas of the sample with those of the calibration standard.

5) Advantages

HPLC allows for the precise quantification of individual anthocyanins, making it superior to spectrophotometric methods, which measure total anthocyanin content without distinguishing between different compounds.

It is highly reproducible and can handle complex food matrices.

This method is essential for accurate anthocyanin analysis in foods such as berries, grapes, and red cabbage, helping in quality control, research, and nutritional studies.

Ultra-High Performance Liquid Chromatography

UHPLC is a powerful analytical tool for separating, identifying, and quantifying anthocyanins in food matrices. Here's a general procedure for analysing anthocyanins using UHPLC:

1. Sample Preparation

Extraction of Anthocyanins:

1) Weigh the food sample (e.g., fruits, vegetables, or beverages).
2) Add acidified methanol (typically 0.1-1% HCl or formic acid in methanol) to the sample in a ratio of around 1:5 to 1:10 (w/v).
3) Homogenize the mixture (e.g., with an ultrasonic bath or homogenizer) for about 15-30 minutes.
4) Centrifuge the sample at around 10,000 rpm for 10-15 minutes to separate the supernatant.

5) To get rid of any particles, pass the supernatant through a membrane filter with a pore size of 0.22 to 0.45 µm

Concentration (optional): If necessary, concentrate the anthocyanins under reduced pressure using a rotary evaporator at low temperatures (e.g., 30-40°C) to avoid degradation.

Dilution: If the extract is too concentrated, dilute it with the mobile phase or water to ensure compatibility with the UHPLC system.

2. UHPLC Conditions

Column: Use a reversed-phase C18 column, which is commonly employed for anthocyanin separation. A column with a small particle size (e.g., 1.7 µm) and a shorter length (50-100 mm) is ideal for UHPLC.

Typical column size: 50 x 2.1 mm or 100 x 2.1 mm, 1.7 µm.

Mobile Phase

Mobile phase A: Acidified water (e.g., 1-5% formic acid, or 1% acetic acid).

Mobile phase B: Acidified organic solvent such as acetonitrile or methanol (with 1-5% formic acid or acetic acid).

Gradient elution

Start with a high percentage of mobile phase A (e.g., 90%) and a low percentage of B (e.g., 10%).

Gradually increase mobile phase B (organic solvent) over 30-40 minutes to about 40-50% to ensure the separation of all anthocyanins.

Flow rate: Typically around 0.2-0.4 mL/min.

Temperature: Maintain the temperature of the column at around 30-40°C.

Detection: Use a photodiode array (PDA) detector at 520-530 nm, which is the typical absorbance range for anthocyanins. Additionally, mass spectrometry (MS) detection can be used for more detailed structural information.

3. Standard Preparation

Prepare calibration standards of known anthocyanins (e.g., cyanidin-3-glucoside, pelargonidin-3-glucoside) by dissolving them in acidified methanol.

Prepare a series of dilutions to create a calibration curve for quantification.

4. Injection

Inject a small volume (e.g., 2-10µL) of the filtered sample into the UHPLC system.

5. Data Analysis

Examine the sample's UV-visible spectra and retention durations in comparison to those of typical anthocyanins.

Construct a calibration curve using standard anthocyanins to quantify the concentration of anthocyanins in the sample.

Optionally, mass spectrometry can be used for confirmation of anthocyanin molecular masses and fragmentation patterns.

Reporting

Depending on the preparation method, report the anthocyanin concentration as mg/100 g of the food sample's fresh weight or dried weight.

This general UHPLC procedure provides precise and reliable analysis of anthocyanins in various food matrices such as berries, wine, and other coloured fruits and vegetables.

Therefore, UHPLC is a powerful analytical tool for separating, identifying, and quantifying anthocyanins in food matrices. Here’s a general procedure for analysing anthocyanins using UHPLC:

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Typical column size: 50 x 2.1 mm or 100 x 2.1 mm, 1.7 µm.

Mobile Phase

Mobile phase A: Acidified water (e.g., 1-5% formic acid, or 1% acetic acid).

Mobile phase B: Organic solvent that has been acidified, like methanol or acetonitrile (with 1-5% acetic or formic acid).

Gradient elution

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Gradually increase mobile phase B (organic solvent) over 30-40 minutes to about 40-50% to ensure the separation of all anthocyanins.

Flow rate: Typically around 0.2-0.4 mL/min.

Temperature: Maintain the temperature of the column at around 30-40°C.

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Prepare a series of dilutions to create a calibration curve for quantification.

4. Injection

Inject a small volume (e.g., 2-10µL) of the filtered sample into the UHPLC system.

5. Data Analysis

Compare the sample's UV-visible spectra and retention periods match those of typical anthocyanins.

Construct a calibration curve using standard anthocyanins to quantify the concentration of anthocyanins in the sample.

Optionally, mass spectrometry can be used for confirmation of anthocyanin molecular masses and fragmentation patterns.

6. Reporting

Report the number of anthocyanins in mg/100 g fresh weight or dry weight of the food sample, depending on the method of preparation.

This general UHPLC procedure provides precise and reliable analysis of anthocyanins in various food matrices such as berries, wine, and other coloured fruits and vegetables.

Thin liquid chromatography

Thin Layer Chromatography (TLC) Procedure for Analysis of Anthocyanins in Food

Materials

1) TLC Plates - Silica gel plates (precoated).
2) Solvents - Typically, a mixture of solvents is used for anthocyanin analysis, such as:
    - Ethyl acetate
    - Methanol
    - Water (can vary depending on the specific anthocyanin type).
    - Formic acid or acetic acid to adjust the pH.
3) Anthocyanin-rich food sample (e.g., berries, grapes, red cabbage, etc.)
4) Mortar and pestle for sample grinding.
5) Solvent chamber - For developing the chromatogram.
6) Capillary tubes or micropipettes - For spotting the sample.
7) UV lamp or lightbox - To visualize the separated components (optional, depending on the detection method).

Procedure

1) Sample preparation

Take a small amount (1-2g) of the food sample and grind it using a mortar and pestle.

Extract anthocyanins using an appropriate solvent. A mixture of methanol and acid (e.g., 1% HCl in methanol) is often used to extract anthocyanins efficiently.

Filter the extract through a filter paper or centrifuge it to remove any solid debris.

2) TLC Plate preparation

Draw a line with a pencil on the silica gel TLC plate, about 1.5 cm from the bottom. For the sample application, this will act as the baseline.

With a capillary tube or micropipette, spot small amounts (2-5 µL) of the filtered sample extract onto the baseline of the TLC plate. Let it dry and repeat a few times to concentrate the sample.

3) Solvent preparation

Prepare the solvent mixture for TLC development. For anthocyanins, an appropriate solvent system is usually:

Solvent A: Water, formic acid, and ethyl acetate (85:10:5)

Solvent B: Water, acetic acid, and butanol (4:1:5).

Adjust the mixture as needed to achieve good separation.

4) Development of TLC Plate

Place the TLC plate into a developing chamber containing the solvent mixture. Make sure the solvent does not touch the sample spots directly, and the chamber is sealed to maintain the vapor.

Allow the solvent to travel up the TLC plate by capillary action until it reaches about 1 cm below the top of the plate.

Mark the solvent front with a pencil as soon as you remove the plate.

5) Visualization

After development, anthocyanins can be visualized due to their natural color (red, purple, blue, depending on pH).

For better visualization, use a UV lamp or a chemical reagent (e.g., ferric chloride or vanillin) to spray on the TLC plate to enhance visibility.

6) Interpretation

Calculate the Rf values, which are the compound's travel distance divided by the solvent front's travel distance.

Each anthocyanin will have a characteristic Rf value.

Compare the Rf values to known standards or reference compounds for anthocyanin identification.

Significance of quantitative analysis of anthocyanins in food

1) **Nutritional value:** Anthocyanins are potent antioxidants, contributing to the health benefits of various fruits and vegetables. Quantifying them helps assess the nutritional quality of food.
2) **Advantages for health:** A lower risk of chronic illnesses like diabetes, heart disease, and some types of cancer has been associated with anthocyanins. Studying their influence on health is made easier with accurate measurement.
3) **Food quality and Authenticity:** The concentration of anthocyanins is a marker of food freshness, ripeness, and quality. Quantitative analysis can also detect adulteration or ensure food authenticity, especially in products like juices and wines.
4) **Shelf-life and Stability:** Anthocyanins degrade over time or due to environmental factors like light and heat. Monitoring their levels can help manufacturers optimize storage and processing conditions to maintain colour and quality.
5) **Regulatory compliance:** In some cases, food products are required to meet certain standards of anthocyanin content for labelling or health claims. Quantitative analysis ensures compliance with these regulations.

Topical analysis of anthocyanins in food

Sophisticated analytical techniques are needed for objective quality control in food production due to the growing need for quality assurance. Although exact, traditional analytical procedures are expensive, time-consuming, labour-intensive, and harmful. Topical methods that don't require reagents, such Near-infrared (NIR) Spectroscopy, Fourier Transform Infrared (FTIR) Spectroscopy, and Raman Spectroscopy, provide a simple, quick, and economical substitute. In the food business, NIRS can be utilized for quality assurance and control. It makes it possible to quickly evaluate a number of factors, including the amount of moisture, fat, protein, and sugar in food

products as well as their acidity, antioxidant content, activity, and micronutrient content, including anthocyanins.

Near infrared spectroscopy for analysis of anthocyanins in food

One practical, non-destructive method for analyzing anthocyanins in food is near-infrared spectroscopy (NIR). Food science and nutrition are interested in anthocyanins because they are pigments that give many fruits and vegetables their red, purple, and blue hues. They also have antioxidant qualities.

Principle of NIR for anthocyanin analysis

NIR spectroscopy works by exposing a sample to near-infrared light (typically between 800 nm to 2500 nm). Molecules within the sample absorb specific wavelengths based on their chemical bonds, especially the bonds C-H, O-H, and N-H, which are present in organic molecules like anthocyanins. The absorbed light provides a spectral signature that is used to analyse the chemical composition of the sample.

Steps in NIR spectroscopy for anthocyanin analysis

1) Sample Preparation

Fresh or processed samples: Fruits, vegetables, or food products containing anthocyanins (e.g., berries, red cabbage, wine) can be analysed directly or after minimal preparation.

Drying or Homogenizing: Some samples may require drying or grinding to ensure consistency and improve accuracy.

2) Data Acquisition

The sample is exposed to near-infrared light, and the NIR spectrometer records the reflected or transmitted light. The absorption of light at specific wavelengths is measured, producing a spectral fingerprint.

3) Spectral Analysis

Chemometric Models: To assess anthocyanin content, chemometric approaches like Partial Least Squares (PLS) regression are used. The reference anthocyanin data from conventional techniques (such as High-Performance Liquid Chromatography, or HPLC) is correlated with the NIR spectra.

Calibration: To forecast the anthocyanin content of unidentified samples, a calibration model is created. The chemometric method and the caliber of the reference data determine how accurate this model is.

4) Quantification

Once calibrated, the NIR method allows for the quick and non-invasive measurement of anthocyanins in dietary items. This method is highly advantageous in routine quality control and food processing environments due to its speed and ease of use.

Advantages of NIR for anthocyanin analysis

- **Non-destructive:** Does not require the destruction of the sample, allowing for repeated measurements.
- **Rapid:** Spectra can be obtained within seconds, making it ideal for high-throughput analysis.
- **Minimal sample preparation:** Often, no complex extraction or hemical treatment is required.
- **Environmentally friendly:** NIR does not involve the use of chemicals or solvents, making it a greener alternative to conventional methods.

Limitations

- **Sensitivity:** While useful for general quantification, NIR is less sensitive than chromatographic techniques (like HPLC) for detecting low levels of anthocyanins.
- **Calibration needs:** Developing robust calibration models requires a large number of samples with known anthocyanin content.
- **Complexity of spectral data:** NIR spectra can be complex, and
- The robustness of the chemometric techniques used determines how well the analysis turns out.

Applications in Food

- **Quality control:** Monitoring anthocyanin levels in processed foods, beverages (e.g., wine, fruit juices), and fresh produce.
- **Breeding and Cultivar selection:** In agriculture, NIR can be used to rapidly screen plant varieties for anthocyanin content.
- **Nutritional analysis:** Assessing the nutritional quality of foods with high anthocyanin content.

In summary, NIR spectroscopy is an useful technique for the analysis of anthocyanins in food, offering a rapid, non-destructive, and environmentally friendly method, though it may require complementary techniques like HPLC for more detailed analysis.

Significance of topical analysis of anthocyanins in food compounds

Topical analysis is important for analysis of anthocyanins in food compounds due to several reasons tied to the specific properties of anthocyanins and the need for accurate assessment of their concentration and distribution in food matrices. Here are the key reasons:

1) Surface Localization of Anthocyanins

Anthocyanins are pigments primarily concentrated in the outer layers (skins, peels, and epidermis) of different vegetables and fruits. For example:

In grapes, anthocyanins are found mainly in the skin, which gives wine its color.

In berries (e.g., blueberries, blackberries), they are concentrated in the outer skin. Therefore, topical analysis targets the specific areas where anthocyanins are most prevalent, providing more accurate information about their distribution and content.

2) Non-Destructive Analysis

Topical analysis, especially when combined with methods like Near-Infrared Spectroscopy (NIR), allows for non-destructive testing of the food's surface, avoiding the need to destroy or alter the sample to measure anthocyanins. This is particularly useful in quality control processes where the integrity of the product needs to be maintained for further processing or sale.

3) Heterogeneous Distribution

Anthocyanins are not evenly distributed throughout the entire food matrix. For example:

In fruits like cherries or apples, the anthocyanins are mainly found in the skin, while the pulp contains little or no pigment. Topical analysis helps focus on the specific areas of interest (the skin or surface) rather than averaging the anthocyanin content across the entire fruit, leading to more precise measurements.

4) Quality Control in Food Processing

In food processing, such as making juices, wines, or jams, the anthocyanin content is a critical factor for color, taste, and nutritional value. Topical analysis ensures that the outer layers, which are often the richest in anthocyanins, are monitored effectively. This helps maintain consistency in product quality.

5) Post-Harvest and Storage Monitoring

The concentration of anthocyanins can change during post-harvest storage and processing due to exposure to light, oxygen, and other factors. Topical analysis allows monitoring of these changes on the surface of foods without needing to damage or alter them. This is crucial for preserving the quality of anthocyanin-rich products.

6) Rapid Screening and Sorting

In agricultural and food industries, topical analysis can be used for rapid screening of large batches of fruits or vegetables based on their anthocyanin content. This is useful for sorting produce, optimizing harvest timing, and selecting varieties with desired pigment levels.

7) Consumer Appeal

Anthocyanins are responsible for the vibrant and differential colouration in many fruits and vegetables. Topical analysis can help ensure that the colour intensity is consistent across batches, which is important for consumer appeal and market value.

8) Health and Nutritional Value

The antioxidant qualities and related health advantages of anthocyanins are well known. Topical analysis makes it possible to assess food's nutritional value and functional qualities more accurately because bioactive substances are primarily present on the food's surface. This guarantees that consumers who are concerned about their health receive the desired nutritional value.

In conclusion, topical analysis is critical for providing accurate, fast, non-destructive measurement of food's anthocyanin concentration, particularly considering its surface location, function in food quality, and health advantages. This approach is particularly valuable in industries like food processing, agriculture, and nutrition, where precision in monitoring anthocyanins directly impacts product quality and consumer satisfaction.

Innovative Spectroscopic Techniques for Food Quality Assurance

The foundation of near-infrared (NIR) spectroscopy is the absorption of electromagnetic radiation with wavelengths between 780 and 2500 nm., is the most widely used method for evaluating food. Infrared (IR) works in the infrared region of the electromagnetic spectrum, which spans from 780 nm to 1000μm [36, 37].

FTNIR spectroscopy usually produces a lesser information power than FTIR spectroscopy because the absorptions of the functional groups are not as

strong as those of the comparable functional groups in the all-infrared regions. The absorptions of -OH (carbohydrate, protein, and lipid) and -NH (protein) groups are better resolved in the NIR than in the IR area, despite the fact that the hydrogen bond interactions cause bigger band shifts than those observed in the IR region, which facilitates their interpretation. Because it offers supplementary information to that derived from FTIR, FTNIR spectroscopy is therefore also dependable for research [38]. Overtones and combinations of basic hydrogenous bond vibrations, such as the many C-H, O-H, S-H, and N-H vibrations, are the foundation of NIR spectroscopy [39]. By combining the NIR spectra with chemometrics, the analytical method known as NIR spectrometry advanced with the technological revolution [40].

In a different study, Miramont *et al*. (2019) created partial least squares (PLS) models using FTIR spectroscopy and chemometrics to forecast the levels of different anthocyanins in red wine throughout the fermentation process. High-performance liquid chromatography with ultraviolet-visible detection was used to measure the concentration of molecular anthocyanins, and the Adams-Harbertson test was used to calculate the ratio of monomeric to polymeric anthocyanins. For both calibration and cross-validation, these authors achieved a high determination coefficient (R2) that was greater than 0.8. For tracking changes in these compounds over time during winemaking, FTIR spectroscopy can be a useful tool for rapid anthocyanin monitoring [41].

IR Characterisation and Identification of Anthocyanins

The most prevalent natural pigments found in plants are anthocyanins, along with betalains, chlorophylls, and carotenoids

The anthocyanin compounds are glycosylated polyhydroxy or polymethoxy derivatives of 2-phenylbenzopyrilium with two benzyl rings or flavylium salts. Five major peaks were identified by Stuppner *et al*. (2020) in their NIR spectroscopy study of the properties of anthocyanins in elderberries: 8600 cm^{-1} (C-H stretching second overtone), 8328 cm^{-1} (C-H stretching second overtone), 6900 cm^{-1} (O-H stretching and N-H asymmetric stretching first overtone), 5620 cm^{-1} (C-H symmetric stretching first overtone), and 5188 cm^{-1} (O-H stretching and deformation combination) [42]. Anthocyanin composition varies among extracts of different origins, according to research by Dai *et al*. (2022) [44]. Dai *et al*. (2022) verified using surface-enhanced Raman spectroscopy that while the anthocyanin extracts [43].

Future perspectives

Further developments in non-destructive testing are anticipated in the future of vibrational spectroscopy in food quality assurance, as research is being conducted to improve the methods' sensitivity, specificity, and mobility. Real-time, on-site analysis of complex spectrum data could be made possible by the combination of artificial intelligence and machine learning. This development demonstrates how NIR and Raman spectroscopy may be used to satisfy the growing standards for food sector quality and safety, guaranteeing the dependability and nutritional value of food products everywhere.

Current challenges in quantitative and topical analytical methods

A broad class of plant pigments known as "natural non-nutritive substances" that are soluble in water and give plants their red hue are called anthocyanins. Flowers, fruits, leaves, stems, and, less frequently, roots and wood all contain these pigments.

. In cells, they are found in vacuoles, in the form of granules of various sizes, while cell walls and pulp tissues do not contain anthocyanins [44]. Determining anthocyanins can provide a number of challenges, many of which are connected to sample preparation prior to analysis. Wet chemical procedures, which entail pigment extraction in organic solvents (such as acetone, methanol, and ethanol) for subsequent spectrophotometric studies, are the conventional method for determining anthocyanins. However, laboratory procedures are laborious, time-consuming, and destructive to the plants [45].

Plant anthocyanins can be identified using traditional (spectrophotometric) or contemporary techniques, such as HPLC in conjunction with different kinds of mass spectrometers or NMR equipment. Nevertheless, spectrophotometric techniques are not enough to precisely describe each anthocyanin found in plant materials. There may be an imprecise relationship between absorbance and the real concentration of anthocyanins. Gitelson *et al.* (2009) shown that there are effective and non-destructive substitutes for wet chemical techniques, such as reflectance and/or absorption spectroscopy. They developed an index for non-destructive estimation of anthocyanins based on the optical properties of leaves and spectral features of anthocyanins [46]. Near-infrared hyperspectral imaging was demonstrated by Martinez-Sandoval *et al.* (2016) to be a quick, affordable, and non-destructive way to screen for anthocyanins in grapes [47]. A quick and highly accurate technique for identifying and visualizing the anthocyanin content of mulberry fruit was presented by Li *et al.* in 2023. Their findings

enable us to draw the conclusion that variations in the anthocyanin content of fruits at various stages of maturity can be seen using hyperspectral imaging [48]. The usefulness of image analysis in determining the number of anthocyanins and other fluorescent phenolic compounds present in strawberry fruit by UV rays was examined by Yoshioka *et al*. (2013) [49]. These findings suggest that genotypes high in anthocyanins can be chosen by image analysis. Expensive laboratory equipment and time-consuming laboratory work can be replaced with rapid picture analysis. Other authors noted that FT-NIR and FT-NIR spectroscopic techniques might be used as quick and non-destructive ways to predict the primary individual anthocyanins and total anthocyanin content in soybean seed.

Advancements and Innovations in anthocyanin analysis

Despite the low cost and non-destructive nature of fast spectroscopy and image analysis techniques, it is important to keep in mind that they require appropriate validation by extractive quantitative analysis.

Rapid evaluation of food safety and quality is becoming more and more necessary these days. Non-invasive technology for evaluating food and the concentration of certain substances must be developed. To enhance and precisely determine the anthocyanin content of foods, a variety of techniques could be tried. For data interpretation, it appears crucial to employ low-cost, user-friendly methods and basic chemometric algorithms. In the future, the application of chemometric artificial intelligence to food analysis appears to be highly intriguing.

Conclusion

In food science, the analytical environment for anthocyanin quantification and characterisation is varied, including both established and new techniques, each with special benefits and drawbacks. The gold standard for in-depth anthocyanin analysis is still High-performance Liquid Chromatography (HPLC), which relies on silica-based reverse-phase columns like C18 and phenyl. The development of new packing technologies such as core-shell columns and the shift to Ultra-high-performance Liquid Chromatography (UHPLC) have greatly improved the speed, resolution, and efficiency of these analyses, making them essential for the accurate identification and quantification of anthocyanins.

Although less advanced, thin-layer chromatography (TLC) is a crucial first step for anthocyanin separation verification and simple profiling. Notwithstanding its ease of use, TLC's combination with cutting-edge detection techniques, including smartphone technology, has created new

opportunities for its use in quality control, especially in environments with limited resources.

The particular goals of the analysis determine which detection methods are used, ranging from UV detectors to Mass Spectrometry (MS) and High-resolution Mass Spectrometry (HRMS). During the profiling stages, UV detectors, such as a photodiode array (PDA) and a diode array detector (DAD), offer important information about the types of anthocyanins. In the meanwhile, MS-particularly HRMS-is preferred due to its unmatched capacity to differentiate between structurally identical anthocyanin molecules, providing a thorough profiling capability.

Spectroscopic techniques like Raman spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, and Near-infrared (NIR) spectroscopy have become popular as quick, easy, and affordable substitutes for anthocyanin analysis and food quality management. A major step towards more approachable food quality assurance procedures is represented by their non-invasiveness, low preparation requirements, and lack of reagent use, all of which are ideal for on-site analysis.

When these approaches are critically examined, it becomes clear that each has unique trade-offs. Despite its analytical strength, HPLC and UHPLC may have operational complexity and cost obstacles that could restrict their use. However, TLC's lesser resolution and difficulties with quantitative analysis limit its usefulness. Despite their potential for quick screening, spectroscopic approaches could not be as sensitive or specific as mass spectrometric or chromatographic procedures. In order to overcome these constraints, a multidisciplinary strategy that makes use of hybrid analytical techniques to capitalize on the advantages of different approaches is required. In order to improve the quality assurance procedures in the food business, future research should concentrate on honing current methods, investigating cutting-edge detection technologies, and creating integrated systems that provide thorough, accurate, and effective anthocyanin analysis.

Researchers are frequently faced with a choice between using topical analysis approaches and quantitative analysis when studying and analyzing the anthocyanins found in food products. Known for its precision and selectivity, quantitative analysis provides a thorough understanding of the presence and changes of anthocyanins in food by precisely measuring their content. But this approach is frequently costly and time-consuming, which makes it a less practical choice for research projects needing quick or wide-ranging insights.

However, topical analysis is notable for being quick and affordable, offering a qualitative summary that allows for a more comprehensive examination of anthocyanin content in a variety of samples, despite its lack of precision. However, topical analysis lacks the specificity and selectivity provided by quantitative methods due to its qualitative nature. This contradiction highlights how important it is to compare different methods to one another in order to strike a balance between the practicality of time and financial restraints and the requirement for precise, in-depth data. A more nuanced understanding of anthocyanins in food can be obtained by combining the two approaches and utilizing their respective advantages to ensure a thorough analysis that takes into account both the breadth of topical observations and the depth of quantitative insights.

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Chapter - 7
Titanium Dioxide (TiO_2) Nanoparticles for Dye Remediation: A Short Review

Authors

Shreyoshi Pal
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Akash Singha Roy
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Aakash Debnath
Department of Biotechnology, School of Life Science, Swami Vivekananda University, Barrackpore, West Bengal, India

Sabyasachi Ghosh
Department of Biotechnology, School of Life Science, Swami Vivekananda University, Barrackpore, West Bengal, India

Chapter - 7

Titanium Dioxide (TiO2) Nanoparticles for Dye Remediation: A Short Review

Shreyoshi Pal, Akash Singha Roy, Aakash Debnath and Sabyasachi Ghosh

Abstract

Wastewater from textile and other industrial processes can contain a variety of dyes, both textile and non-textile, contributing to water pollution. The effects of textile dyes (like- direct dyes reactive dyes, sulfur dyes etc.) and nontextile dyes (like- food industry dyes and leather industry dyes, etc.)on water pollution are multi-faceted and can have various environmental and ecological consequences: such as Chemical Contamination, Reduced Light Penetration, Affects Aquatic Organisms, Biomagnification, Disturbance of Biodiversity, Pollution, Groundwater Contamination, etc. Nanotechnology offers promising solutions for the degradation of dyes. Several nanomaterials like Titanium Dioxide (TiO2), Silver Nanoparticles (AgNPs), Iron Oxide Nanoparticles (Fe_2O_3, Fe_3O_4), Carbon Nanotubes (CNTs), Zinc Oxide (ZnO), Polymeric Nanomaterials (e.g., Nanocomposites), etc., have been explored for their effectiveness in the degradation of dyes through various mechanisms, including photocatalysis, advanced oxidation processes (AOPs), and other catalytic reactions. Here we are mainly focusing on Titanium Dioxide (TiO2) nanoparticles because of their ability of degrade of dyes by their photo-catalytic activity and make wastewater reusable.

Keywords: Nanotechnology, dyes, pollution

Introduction

The discharge of visual pollutants from industries, including the textile industry& non-textile industries can have aesthetic, environmental, and public health implications. Often these industries release pollutants to the neighboring environment without any purification. These contaminants seriously poison aquatic life and other creatures in addition to colouring the water. The overuse of dyes in numerous industries, such as non-textile and textile industries, can indeed lead to the significant presence of dye compounds in wastewater. When not properly managed or treated, the effluent

stream from these industries may contain a considerable percentage of residual dyes. This poses environmental challenges due to the potential toxicity, persistence, and visual impact associated with many synthetic dyes. Dyes are bulky complex organic molecules that can attach themselves to substances like cloth or paper etc. Dyes play a crucial role in adding aesthetic appeal to a wide range of products and are extensively used in different industries. However, textile or nontextile industries' outflow is harmful to humans, aquatic life, and the overall environment.

Toxicity of textile and nontextile dyes: Toxicity is the capability of harming the environment and humans. Textile and non-textile dyes can affect the environment by following:

a) Dyes are composed of complex chemicals, organic substances, heavy metals etc., when discharged into water can lead to chemical contamination or mixing of complex substances into water, thus causing a harmful effect.

b) Certain dye molecules can persist in water and reduce light penetration. This can negatively affect photosynthesis in aquatic plants and algae, disrupting the balance of the aquatic ecosystem and impacting the overall health of the water body.

c) Dyes can be harmful to aquatic life as they are toxic, complex chemical or organic substances. They can create various toxicological reactions like growth inhibition, abnormal pigmentation, health hazards, etc.

d) Dyes bioaccumulate in waters, causing environmental degradation, destruction, and manipulation of microbial populations. The dyes remain in the environment and diffuse into the food source, causing greater contamination in a food chain, and leading to biomagnification.

e) The release of wastewater containing toxic substances such as dyes can cause changes in microbial diversity by inhibiting the growth of certain microbes.

f) Many industrial wastewaters can damage the quality of soil and plants and the quality of agricultural fields when it is used for irrigation.

g) Use of dye-contaminated groundwater can cause severe health problems and, in some cases, death. Dyes contained in wastewater enter aquatic animals, travel through the food source, and eventually

reach humans, causing high blood pressure, fever, kidney disease, stomach pain, and other problems in the body.

h) The dyeing industry causes pollution by emitting CO_2, NO_2, SO_2, and other gas emissions during production also gallons of water and toxic chemical dyes are used and released to local areas. During the flow of sewage, many harmful substances enter the soil, destroying soil, groundwater, ponds, and plants, making the soil and water toxic.

Dye degradation using TiO2 NPs: TiO2 NPs are widely investigated for their ability to dye removal from aqueous solutions. The use of TiO2 nanoparticles in dye removal is often based on their photocatalytic properties, where the nanoparticles, when exposed to ultraviolet light, can generate ROS and initiate the degradation of organic pollutants, including dyes. Titanium dioxide (TiO2) nanoparticles are particles having diameters lesser than 100 nm. As they are very small in size and have a large surface area, they can very effectively absorb and react. TiO2 NPs are light-activated catalytic molecules, which not only activate under UV radiation but also work under visible light. When irradiated with UV light it can oxidize the dyes into much smaller structures. The photocatalytic mechanism of TiO2 is shown in Figure 1.

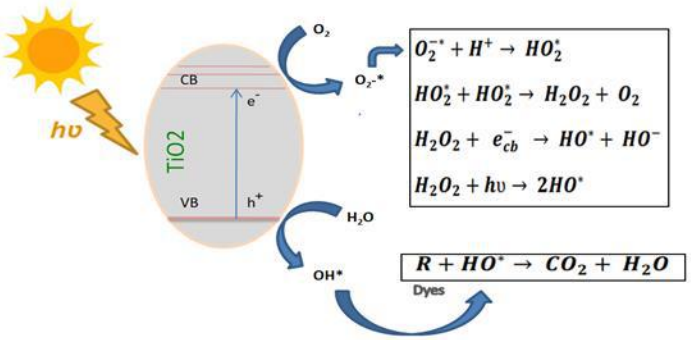

Fig 1: Photocatalytic mechanism of TiO2.

In the presence of light energy, the electron in the valence bond of the TiO2 semiconductor gets excited and jumps from the valence band to the higher energy conduction band. It occurs when the incident light energy is higher than the band gap energy so that the transition of electrons is possible. Upon transition, the conduction band is now electron-rich and the valence band shows comparative electron deficiency so that holes are produced in the valence band. Now electrons in the conduction band participate in the interaction in the vicinity of oxygen to produce superoxide radicals (O2-) while on the other hand holes in the valence band interact with the

neighborhood water molecule to produce hydroxyl radicals (OH*). These free radicals will interact with the hydrophilic organic compounds or polymers in their surroundings. Hydrogen peroxide H_2O_2 is produced when superoxide radicals (O_2-) react with water (H_2O). Then this H_2O_2 interacts with electrons and produces reactive hydroxyl radicals, In the presence of light energy (hυ) hydrogen peroxide (H_2O_2) will convert to hydroxyl radicals (OH*). These hydroxyl radicals (OH*) are now eligible to degrade the organic polymer or in this case textile and non-textile dyes(R) into water (H_2O) and carbon dioxide (CO_2). The photocatalytic mechanism of TiO2 can be explained as follows

Future Trend

TiO2 NPs are highly photoactive catalytic agent, with their affordable price, and stability, they can be a hope for the future degradation of dyes. Furthermore, TiO2 is widely available easy to reach, and less toxic so it makes sense to use titanium dioxide (TiO2) nanoparticles to degrade dyes from wastewater and make dye-integrated water reusable. Despite having several advantages such as a small size large, surface area, highly reactive, less costly, etc the technology is still under development and still needs to be implemented at the industrial level.

Conclusion

The many textiles and nontextile sectors generate a variety of wastewater types that contain dyes. These dyes have extremely complex toxicological impacts on the environment and on living things, ranging from simple single-cellular creatures or microorganisms to sophisticated human life. Thus, it is very essential to degrade the dye by removing the dye out from wastewater reducing water pollution and making it reusable. Here come titanium dioxide (TiO2) nanoparticles to the rescue, because TiO2 exhibits its catalytic efficiency in the presence of light, and requires low cost to buy. It is a photostable and biologically stable compound having low toxicity so, they can very easily degrade dyes in the presence of UV or visible light. Thus, we can use titanium dioxide (TiO2) nanoparticles to treat and purify the dye-containing wastewater.

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Chapter - 8
Transforming Neurological Therapies: The Biotechnological Impact of Neuromodulation

Authors

Priyanka Mandal
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Bidisha Ghosh
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Subhasis Sarkar
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Suranjana Sarkar
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Chapter - 8

Transforming Neurological Therapies: The Biotechnological Impact of Neuromodulation

Priyanka Mandal, Bidisha Ghosh, Subhasis Sarkar and Suranjana Sarkar

Abstract

Neuromodulation is a cutting-edge area within biotechnology that involves delivering targeted electrical or chemical stimuli to specific neural sites to modulate nerve activity. This innovative approach aims to treat various chronic conditions and neurological disorders by precisely influencing nervous system functions. Techniques such as deep brain stimulation (DBS), spinal cord stimulation (SCS), and transcranial magnetic stimulation (TMS) have demonstrated significant efficacy in managing disorders like Parkinson's disease, chronic pain, epilepsy, and depression. Neuromodulation offers a customizable and often reversible alternative to traditional pharmacological treatments, providing symptom relief where conventional therapies may not succeed. Advancements in biotechnology are enhancing the precision, effectiveness, and safety of neuromodulation. Research efforts are focused on creating advanced closed-loop systems that can automatically adjust stimulation parameters in real-time based on neural feedback. Moreover, novel bioengineering methods are improving the integration of neuromodulation devices with the body’s neural networks, leading to better patient outcomes. As our understanding of neural circuits and their role in various diseases deepens, neuromodulation has the potential to transform the treatment landscape for numerous neurological and psychiatric disorders, offering new hope and improved quality of life for patients with challenging conditions.

Keywords: Bioengineering, deep brain stimulation (DBS), electrophysiology, neural stimulation, neuromodulation, neurotherapeutics

Introduction

Neurological disorders, such as epilepsy, Parkinson's disease, chronic pain, and depression, present significant challenges to healthcare systems worldwide, impacting millions and often leading to severe consequences.

Traditional treatments, which typically involve medications and surgical procedures, often provide limited relief and can have considerable side effects. In response, neuromodulation has emerged as a transformative approach, offering innovative methods to modulate nervous system activity and provide targeted treatments for these complex conditions. Neuromodulation involves applying electrical or chemical stimuli to specific neural sites to modify nerve activity and achieve therapeutic outcomes. Techniques such as deep brain stimulation (DBS), spinal cord stimulation (SCS), and transcranial magnetic stimulation (TMS) have demonatrated considerable efficiency in treatment of various disorders, including Parkinson's disease, chronic pain, epilepsy, and depression. Unlike traditional pharmacological treatments, neuromodulation offers a customizable and often reversible method of symptom relief, particularly in cases where conventional therapies have failed (Johnson *et al*., 2013).

Deep brain stimulation (DBS) involves implanting electrodes into specific brain regions, such as the subthalamic nucleus or globus pallidus, which are connected to an implanted pulse generator (IPG) that continuously delivers electrical impulses. This technique has been useful in lessening motor symptoms in Parkinson's disease patients and is being explored for other conditions like obsessive-compulsive disorder (OCD) and Tourette syndrome. Although the precise mechanisms by which DBS works are still being studied, it is believed to modulate abnormal neural circuits and restore normal neural activity patterns (Lozano *et al*., 2019).

Spinal cord stimulation (SCS) involves placing electrodes in the epidural space of the spinal cord, typically at the thoracic or lumbar levels. These electrodes deliver electrical pulses that interrupt pain signal transmission to the brain. SCS has been effective in managing chronic pain conditions, such as failed back surgery syndrome and complex regional pain syndrome, by modulating pain pathways and altering pain perception. The development of advanced waveforms and stimulation paradigms, including burst and high-frequency stimulation, has further enhanced SCS's effectiveness (Aryal *et al*., 2019).

Transcranial magnetic stimulation (TMS) is a non-invasive technique that uses electromagnetic induction to generate electrical currents in targeted brain regions. Repetitive TMS (rTMS) has been approved for treating major depressive disorder and is being investigated for other conditions, such as schizophrenia and stroke rehabilitation. TMS can modulate cortical excitability and plasticity, influencing neural networks involved in mood regulation and cognitive functions. Recent advancements in coil design and

targeting algorithms have increased TMS's precision and effectiveness. Advancements in biotechnology are crucial in improving the precision, effectiveness, and safety of neuromodulation therapies. Research efforts are focusing on developing advanced closed-loop systems that can automatically adjust stimulation parameters in real-time based on neural feedback, thereby optimizing therapeutic outcomes. Closed-loop systems use feedback signals, such as local field potentials or electromyographic activity, to dynamically adjust stimulation settings, maximizing therapeutic effects while minimizing side effects (Mishra *et al*., 2011; Chail *et al*., 2021). Additionally, novel bioengineering techniques are enhancing the integration of neuromodulation devices with the body's neural networks. This includes developing biocompatible materials, flexible electrodes, and wireless power transfer systems, which improve the longevity and functionality of implanted devices. Techniques such as optogenetics, which use light to control genetically modified neurons, and chemogenetics, which employ engineered receptors activated by designer drugs, are expanding the toolkit for neuromodulation, offering new ways to precisely manipulate neural circuits. As our understanding of neural circuits and their roles in various diseases deepens, the potential for neuromodulation to revolutionize the treatment landscape for neurological and psychiatric disorders becomes increasingly evident.

Deep Brain Stimulation

Deep brain stimulation (DBS) is a surgical procedure that involves implanting electrodes in targeted brain regions to deliver continuous electrical impulses. These impulses modulate abnormal neural activity, offering therapeutic benefits for various neurological and psychiatric conditions. The procedure involves the following steps (Pycroft *et al*., 2018; Krauss *et al*., 2021)

The detailed procedure is explained below (Senevirathne *et al*., 2023; Hariz *et al*., 2022):

Implantation of Electrodes

The procedure begins with the surgical placement of thin, insulated wires called electrodes into targeted areas of the brain. The specific regions depend on the condition being treated:

In the treatment of Parkinson's disease, electrodes are typically implanted in the subthalamic nucleus (STN) or the globus pallidus internus (GPi). For essential tremor, the usual target is the ventral intermediate nucleus (VIM) of the thalamus. For obsessive-compulsive disorder (OCD) and other conditions, different targets are selected based on the affected neural circuitry.

Connection to a Pulse Generator

These electrodes are connected to an implanted pulse generator (IPG), also known as a neurostimulator, which is usually positioned under the skin near the collarbone or in the abdomen. The IPG is a small, battery-powered device that sends electrical impulses through the electrodes to the brain.

Delivery of Electrical Stimulation

The IPG delivers continuous electrical pulses to the brain, adjustable in:

- **Frequency:** The number of pulses per second.
- **Amplitude:** The strength or intensity of the electrical current.
- **Pulse width:** The duration of each pulse.

Modulation of Neural Activity

The electrical stimulation modulates neural activity in the targeted brain regions. It is believed that DBS affects the neural circuits involved in the disorder. Potential mechanisms include:

- **Inhibition:** Reducing overactivity in specific neural circuits, which is beneficial in conditions like Parkinson's disease and essential tremor.
- **Excitation:** Enhancing activity in underactive areas, which may be useful for conditions like depression.
- **Disruption of pathological patterns:** Interrupting abnormal neural firing patterns and resetting neural activity to more normal levels.

Adjustment and Optimization

After implantation, the settings of the DBS device are carefully adjusted to maximize therapeutic benefits while minimizing side effects. This process, known as programming, involves multiple visits to a neurologist or a specialized DBS team. Together, patients and clinicians fine-tune the device settings based on symptom relief and any side effects experienced.

Reversible and Adjustable

A significant advantage of DBS is its reversibility and adjustability. Unlike lesions or other permanent surgical interventions, DBS does not destroy brain tissue. The stimulation parameters can be modified over time to adapt to changes in the patient’s condition or to address any emerging side effects.

Deep Brain Stimulation (DBS) has been shown to be beneficial for a variety of medical conditions. It is notably effective in managing Parkinson's Disease, where it helps reduce motor symptoms such as tremors, rigidity, and bradykinesia. DBS is also used to treat Essential Tremor, particularly in cases where medication is ineffective. In patients with Dystonia, DBS can improve severe muscle contractions and movement issues. For individuals with Obsessive-Compulsive Disorder (OCD) who do not respond to standard treatments, DBS has been found to provide symptom relief. Moreover, DBS can be helpful in reducing the frequency and intensity of seizures in certain individuals with Epilepsy.

Spinal cord stimulation

Spinal cord stimulation (SCS) is a therapeutic technique used to manage chronic pain and certain neurological conditions by influencing neural activity in the spinal cord (Sdrulla *et al*., 2018). A detailed description is provided below (Hussaini *et al*., 2017; Traeger *et al*., 2023):

Device components

- **Electrodes:** These are thin wires with electrical contacts that are implanted in the epidural space of the spinal cord.
- **Pulse generator:** A small device, similar to a pacemaker, is implanted under the skin, usually in the abdomen or buttock.

Mechanism of Action

- The pulse generator delivers electrical pulses to the spinal cord through the electrodes.
- These electrical impulses disrupt pain signals before they reach the brain, effectively altering or masking them.
- Although the precise mechanism is not entirely clear, it is believed to involve the inhibition of pain pathways and the activation of inhibitory neural circuits.

Application

Spinal cord stimulation (SCS) is mainly utilized to manage chronic pain that does not respond to other treatments. It has proven effective for conditions such as failed back surgery syndrome (FBSS), complex regional pain syndrome (CRPS), peripheral neuropathy, arachnoiditis, and ischemic limb pain. SCS is especially useful for neuropathic pain, which arises from nerve damage. Additionally, ongoing research is investigating the use of SCS for movement disorders like Parkinson's disease and spasticity related to spinal

cord injuries. Furthermore, SCS can improve blood flow and alleviate pain in patients with peripheral vascular disease, potentially preventing the need for limb amputation.

Transcranial Magnetic Stimulation (TMS)

Transcranial magnetic stimulation (TMS) is a sophisticated technique used to influence brain activity non-invasively, providing therapeutic benefits for various neurological and psychiatric conditions. Here's an in-depth look at how TMS works and its underlying mechanisms:

Fundamental Principles

Electromagnetic induction: TMS operates based on Faraday's law, where a changing magnetic field induces an electric current in a conductor. In TMS, the conductor is the brain tissue.

Magnetic coil: A coil of wire in the TMS device generates a magnetic field when a high-intensity electric current passes through it. This magnetic field is directed perpendicular to the coil's plane.

Magnetic pulses: The magnetic field is delivered in short pulses, which can be single, paired, or repetitive, depending on the intended effect.

Interaction with the Brain

Magnetic field penetration: The magnetic field easily penetrates the skull, reaching the brain tissue without significant attenuation, allowing for non-invasive stimulation.

Induced electric current: When the magnetic field reaches the brain, it induces an electric current within the neurons. This current flows parallel to the coil's plane and follows the path of least resistance, typically along the cortical surface and neural pathways.

Effects on Neurons

Neuronal activity: The induced electric current can depolarize or hyperpolarize neuronal membranes.

Depolarization: This occurs when the membrane potential is brought closer to the threshold, triggering an action potential or neural firing.

Hyperpolarization: This makes neurons less likely to fire by moving the membrane potential away from the threshold.

Excitatory and Inhibitory effects: The effects depend on the frequency and pattern of the TMS pulses:

- High-frequency TMS (≥5 Hz) - typically increases neural activity.
- Low-frequency TMS (≤1 Hz) - typically decreases neural activity.

Therapeutic Mechanisms

Transcranial magnetic stimulation (TMS) has several therapeutic mechanisms. It can regulate abnormal cortical excitability by boosting activity in underactive brain regions, which is particularly useful for depression treatment. TMS also influences cognitive and emotional processes by modulating specific brain regions and their networks, benefiting conditions like depression, anxiety, and obsessive-compulsive disorder (OCD). Additionally, TMS impacts pain perception by targeting neural circuits involved in pain processing, such as the primary motor cortex and descending pain pathways. Furthermore, TMS promotes the growth of new neurons and enhances synaptic plasticity, leading to better cognitive functions and mood regulation. These mechanisms collectively make TMS effective for various neurological and psychiatric conditions. Transcranial magnetic stimulation leverages electromagnetic principles to modulate brain activity, offering promising therapeutic potential for a variety of neurological and psychiatric disorders. By influencing brain connectivity and function, TMS represents a powerful tool in modern neuromodulation (Huang *et al*., 2022).

Current Status

Deep brain stimulation (DBS), spinal cord stimulation (SCS), and transcranial magnetic stimulation (TMS) each play crucial roles in treating a variety of medical conditions, with ongoing advancements enhancing their effectiveness and applications. DBS is predominantly used for managing Parkinson's disease, essential tremor, dystonia, epilepsy, and severe cases of obsessive-compulsive disorder (OCD). Recent innovations in DBS include the development of closed-loop systems that adjust stimulation based on real-time feedback, as well as improvements in device design and battery life. Despite these advancements, DBS remains an invasive procedure with potential risks and variable effectiveness, including side effects such as speech difficulties and balance issues. SCS is primarily applied to manage chronic pain conditions such as failed back surgery syndrome (FBSS), complex regional pain syndrome (CRPS), and neuropathic pain, and it also helps improve blood flow in peripheral vascular disease. Newer SCS techniques involve high-frequency stimulation that avoids the tingling sensations associated with traditional methods, burst stimulation for specific pain types, and customized programming to better address individual patient needs. The challenges with SCS include the risks associated with surgery, variable pain relief outcomes, and issues like lead migration that may necessitate additional procedures.

TMS, a non-invasive method, is effective for treatment-resistant depression and shows growing evidence of usefulness for generalized anxiety disorder (GAD), post-traumatic stress disorder (PTSD), OCD, chronic pain, and migraines. Advancements in TMS include personalized treatments guided by neuroimaging to refine stimulation targets, faster theta burst stimulation protocols, and ongoing research into its potential for conditions such as schizophrenia, bipolar disorder, and cognitive enhancement in neurodegenerative diseases. Although TMS is generally well-tolerated, it faces challenges related to cost, variability in patient response, and occasional mild side effects like scalp discomfort and headaches, with rare instances of seizures. DBS, SCS, and TMS are continually evolving with improvements that enhance their clinical applications. DBS and SCS involve surgical procedures with associated risks but offer significant benefits for movement disorders and chronic pain. TMS provides a non-invasive alternative with promising results for various psychiatric and neurological conditions, though optimizing its use and improving accessibility are ongoing goals.

Conclusion

Deep brain stimulation (DBS), spinal cord stimulation (SCS), and transcranial magnetic stimulation (TMS) are advanced treatments with distinct mechanisms for addressing neurological and psychiatric disorders. DBS is effective for conditions like Parkinson's disease and severe OCD but requires invasive surgery, which comes with risks and variable results. SCS is used for managing chronic pain and improving blood flow in peripheral vascular disease through advanced stimulation techniques, though it also involves surgical risks and potential complications such as lead migration. TMS, a non-invasive approach, shows promising results for various conditions, including depression and chronic pain, but faces challenges related to cost, effectiveness variability, and minor side effects. Ongoing advancements in these therapies are likely to enhance their precision, personalization, and accessibility, ultimately improving treatment outcomes and patient care.

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Chapter - 9
Epigenetic Modulation: Harnessing Gene Expression Therapeutic Potential

Authors

Santanu Mishra
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Bidisha Ghosh
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Subhasis Sarkar
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Suranjana Sarkar
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Chapter - 9

Epigenetic Modulation: Harnessing Gene Expression Therapeutic Potential

Santanu Mishra, Bidisha Ghosh, Subhasis Sarkar and Suranjana Sarkar

Abstract

Epigenetic editing is an innovative approach that enables the precise modification of the epigenome to regulate expression without altering the sequence of DNA. This method uses engineered proteins, such as CRISPR/Cas9 combined with epigenetic modifiers, to target specific genomic regions and alter epigenetic marks like DNA methylation and histone modifications. Epigenetic editing holds significant potential for correcting abnormal gene expression patterns linked to diseases such as cancer, neurological disorders, and metabolic conditions. By providing a highly specific and reversible means of controlling gene activity, epigenetic editing offers new avenues for therapeutic interventions and individualized medicine. Initial research has demonstrated success in reactivating silenced tumor suppressor genes, lowering oncogene expression, and affecting disease-related pathways, highlighting its potential for therapeutic applications. As research progresses, it will be essential to address challenges related to delivery efficiency, off-target effects, and long-term stability. The ongoing development and refinement of epigenetic editing technologies could transform the treatment of complex diseases, providing precise and adaptable strategies for gene regulation and disease management.

Keywords: CRISPR/Cas9, Disease therapy, epigenetic editing, gene regulation, precision medicine

Introduction

Epigenetic regulation is essential for controlling gene expression without altering the DNA sequence. This process involves various chemical modifications such as DNA methylation, histone modifications, and non-coding RNAs, which together form the epigenome. These epigenetic marks are essential for numerous biological processes, such as development, differentiation, and environmental response. Disruptions in epigenetic modifications can lead to abnormal gene expression, contributing to diseases such as cancer, neurological disorders, and metabolic conditions. Traditional

methods to correct gene expression typically involve altering the DNA sequence through gene therapy and genome editing. These techniques can be irreversible and carry risks such as unintended mutations and off-target effects. Epigenetic editing, on the other hand, offers a precise approach to modulating gene expression by directly modifying epigenetic marks (Loscalz and Handy *et al*., 2014; Li, 2021). This emerging technology utilizes engineered proteins, including the CRISPR/Cas9 system combined with epigenetic modifiers, to target specific genomic regions and adjust their epigenetic state. This allows for controlled and reversible activation or repression of gene expression. The CRISPR/Cas9 system, adapted from a bacterial immune mechanism, has become a powerful tool for genome editing in mammalian cellsWhen combined with epigenetic modifiers such as DNA methyltransferases (DNMTs), ten-eleven translocation enzymes (TETs), or histone acetyltransferases (HATs), CRISPR/Cas9 can enable site-specific epigenetic modifications. For example, fusion of dCas9 (a catalytically inactive Cas9) with DNMT3A can induce targeted DNA methylation, while fusion with TET1 can promote targeted DNA demethylation (Karlson *et al*., 2021). Similarly, dCas9 fused to HATs like p300 can enhance histone acetylation at specific loci, boosting gene expression (Cai *et al*., 2023). Developing more advanced and reliable delivery methods, such as viral vectors, lipid nanoparticles, and exosomes is essential for the clinical application of epigenetic editing technologies.

Epigenetic editing holds transformative potential for various applications. It can potentially reactivate silenced tumor suppressor genes in cancer, decrease oncogene expression, and correct gene expression defects in various diseases. Early studies have shown promising results, demonstrating significant therapeutic benefits in preclinical models. For instance, targeted demethylation of the MLH1 gene promoter (Tang *et al*., 2012) has been shown to reactivate its expression in colorectal cancer cells, restoring DNA mismatch repair function and reducing tumorigenicity.

Mechanistic overview

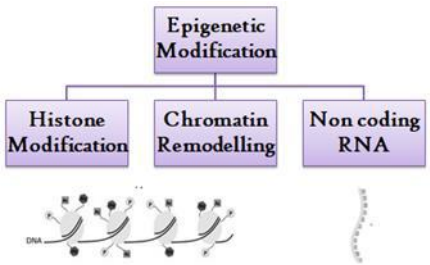

Fig 1: Epigenetic regulation

Histone Modifications

Histone tails experience various post-translational modifications such as acetylation, methylation, phosphorylation, ubiquitination, and sumoylation. Specific enzymes dynamically add and remove these modifications.

DNA Methylation

DNA methylation involves the addition of a methyl group to the 5' carbon of the cytosine ring, creating 5-methylcytosine (5mC). DNA methyltransferases (DNMTs) catalyze this reaction. DNMT1: Maintains methylation patterns during DNA replication:

DNMT3A and DNMT3B: Establish new methylation patterns during development and in response to environmental changes.

CpG Islands: CpG islands are regions rich in CpG dinucleotides, frequently found near gene promoters. Methylation of CpG islands in these promoter regions generally leads to gene silencing.

Histone Methyltransferases (HMTs): Add methyl groups to lysine or arginine residues. The effect of methylation can vary:

- H3K4me3 (tri-methylation of lysine 4 on histone H3) linked with gene activation.
- H3K27me3 (tri-methylation of lysine 27 on histone H3) linked with gene repression.
- **Histone Demethylases (HDMs):** Removes methyl groups from histones, reversing the effects of methylation.

Functional Impact

Transcriptional Repression

Methylated DNA can directly inhibit the binding of transcription factors to promoters. Methyl-CpG-binding domain proteins (MBDs) identify methylated CpG sites and recruit chromatin remodeling complexes, histone deacetylases (HDACs), and other co-repressors, leading to a compacted chromatin state and transcriptional silencing.

Genomic Imprinting and X-Inactivation

Methylation has a critical function in genomic imprinting; only a single allele of a expressed based on the parent origin. X-inactivation in females, where one of the X chromosomes is silenced, involves extensive DNA methylation.

DNA Acetylation

Key Enzymes

Histone Acetyltransferases (HATs): Adds acetyl groups to lysine residues, neutralizing their positive charge and decreasing histone-DNA interactions, leading to a more open chromatin structure and active transcription.

Histone Deacetylases (HDACs): Remove acetyl groups, leading to chromatin condensation and gene expression inhibition.

Functional impact

Gene activation and Repression

- Acetylation generally correlates with gene activation by making chromatin more accessible to transcription factors.
- Methylation can either activate or repress genes, depending on which specific residues are modified.

Epigenetic Memory

- Histone modifications can be inherited through cell division, providing a mechanism for maintaining gene expression patterns across generations of cells.

Non-Coding RNAs (ncRNAs)

MicroRNAs (miRNAs)

- miRNAs are short RNAs, approximately 22 nucleotides in length, that bind to complementary sequences in the 3' untranslated region (UTR) of target mRNAs.
- This binding leads to mRNA degradation or translational repression, thereby downregulating gene expression.

Long Non-Coding RNAs (lncRNAs)

- lncRNAs are longer than 200 nucleotides and can interact with DNA, RNA, and proteins.
- They can recruit chromatin-modifying enzymes to specific genomic loci, affecting the local chromatin structure and gene expression.

Small Interfering RNAs (siRNAs)

- siRNAs are similar to miRNAs but typically arise from double-stranded RNA and are involved in RNA interference (RNAi)

pathways, leading to the degradation of complementary mRNA targets.

Functional Impact

Regulation of Gene Expression

- ncRNAs play roles in fine-tuning gene expression, responding to developmental cues, and stress signals.

Chromatin organization

- lncRNAs can guide chromatin-modifying complexes to specific genomic regions, influencing the 3D organization of the genome and gene expression.

Chromatin Remodeling

ATP-Dependent Chromatin Remodelers

- These complexes use the energy from ATP hydrolysis to alter the position or composition of nucleosomes.
- Major families include SWI/SNF, ISWI, CHD, and INO80.

Actions

- Nucleosome Sliding: Moving nucleosomes along the DNA to expose or occlude regulatory regions.
- Nucleosome Ejection: Removing nucleosomes to make DNA more accessible.
- Histone Variant Exchange: Replacing standard histones with histone variants that have different regulatory properties.

Functional Impact

Gene Accessibility

Chromatin remodeling is crucial for allowing access to DNA during transcription, replication, and repair processes.

Regulation of development and differentiation

Remodeling complexes play key roles in cell lineage commitment and differentiation by altering chromatin structure to activate or repress specific gene programs.

Application of epigenetic regulations

Epigenetic regulations, which involve changes like DNA methylation, histone modifications, and non-coding RNAs, influence gene activity without

changing the DNA sequence. These changes play crucial roles in various biological processes and disease mechanisms, offering applications across medicine, agriculture, environmental science, and biotechnology. In medicine, targeting epigenetic changes is key for cancer therapies, using drugs like DNA methyltransferase inhibitors and histone deacetylase inhibitors to reverse abnormal modifications and reactivate tumor suppressor genes. Additionally, epigenetic mechanisms are linked to neurological disorders such as Alzheimer's, autism, and schizophrenia, paving the way for new therapeutic approaches. Epigenetic reprogramming also holds promise in regenerative medicine by converting differentiated cells into induced pluripotent stem cells for tissue engineering. In agriculture, epigenetic modifications can enhance crop traits like yield, stress resistance, and nutritional value. For example, altering DNA methylation patterns can improve plant responses to environmental stresses such as drought or salinity. Plant breeders also use epigenetic markers to efficiently select for specific traits, speeding up the development of improved crop varieties. In environmental science, epigenetic regulations assist in bioremediation by enhancing microorganisms' abilities to degrade pollutants. By modifying gene expression in metabolic pathways, engineered microbes can be optimized for environmental cleanup. Additionally, epigenetic changes help organisms adapt to changing environmental conditions, providing insights into how species respond to climate change (Wang *et al*., 2022). In biotechnology, epigenetic tools are used in synthetic biology applications, such as developing biosensors and biofactories for pharmaceutical production. Coupled with technologies like CRISPR-Cas9, epigenetic modulators allow precise control of gene expression without altering the DNA sequence, advancing research and therapeutic gene editing. In personalized medicine, epigenetic modifications serve as biomarkers for various diseases, including cancers and autoimmune disorders, aiding early diagnosis, prognosis, and treatment monitoring (Tan *et al*., 2022). Epigenetic inheritance, where changes are passed across generations without altering DNA sequences, offers new ways to study the long-term effects of environmental exposures, diet, and lifestyle on health and disease. Overall, epigenetic regulations offer transformative solutions across multiple fields. In medicine, they provide new therapeutic targets and diagnostic tools; in agriculture, they offer strategies for crop improvement and sustainability; in environmental science, they enhance bioremediation and ecological adaptation; and in biotechnology, they drive innovations in synthetic biology and gene editing. As our understanding of epigenetics deepens, its applications will continue to expand, addressing complex biological challenges and driving future advancements (Wu *et al*., 2023).

Epigenetic editing

Epigenetic editing technologies are revolutionizing our ability to control gene expression without altering the DNA sequence itself. CRISPR/Cas9-based systems, including modified versions like dCas9, enable precise targeting of specific genomic regions to add or remove epigenetic marks, effectively regulating gene expression. These modified Cas9 variants can be fused with epigenetic modifiers such as TET1, DNMT3A, p300, or KRAB to achieve targeted demethylation, methylation, histone acetylation, or histone deacetylation, respectively. Emerging tools like base editors and prime editors offer further precision by making specific nucleotide changes that indirectly influence epigenetic states, converting cytosine to thymine or adenine to guanine without causing double-strand breaks, which reduces off-target effects. In developmental biology, epigenetic modifications are crucial for guiding stem cell differentiation into various cell types during embryogenesis (Lo and Ki., 2017). Specific epigenetic signatures maintain tissue identity and function, ensuring proper development and cellular function (Ueda *et al*., 2023). Epigenomics and systems biology use epigenome-wide association studies (EWAS) to identify epigenetic modifications linked to diseases or traits at the population level. Integrating epigenomic data with genomics, transcriptomics, and proteomics provides a comprehensive understanding of biological systems and their regulation. Environmental epigenetics explores how factors like diet, toxins, stress, and lifestyle affect epigenetic modifications and gene expression, leading to the development of epigenetic biomarkers for assessing environmental exposures and their health impacts. Research on aging and epigenetics focuses on epigenetic clocks that predict biological age based on DNA methylation patterns and the role of age-related epigenetic changes in age-associated diseases and conditions (Jim and Lin, 2022). The ethical, legal, and social implications (ELSI) of epigenetic research and therapies are significant. Ethical considerations include the potential for unintended consequences and the need for informed consent, while legal and regulatory aspects address policies for the safe and equitable use of epigenetic therapies (Sasaki-Honda *et al*., 2023). Social implications involve the impact of epigenetic findings on public health strategies and personalized medicine. Advances in epigenetic therapies include next-generation drugs targeting non-coding RNAs and chromatin remodelers, as well as the potential for combining these drugs with other treatments to enhance therapeutic outcomes.

Epigenetic studies in non-human systems, such as animal models like mice and zebrafish, provide insights into the mechanisms underlying human diseases. In plant biology, epigenetics influences development, adaptation,

and breeding, affecting traits such as yield, stress resistance, and nutritional value. Future research directions include the role of 3D genome organization in gene regulation and the impact of the microbiome on the host epigenome (Hörnblad and Remeseiro, 2022). Technological innovations, such as single-cell epigenomics and high-throughput sequencing, continue to advance the field, offering new tools for analyzing epigenetic modifications with greater precision and depth. Incorporating these aspects will provide a comprehensive overview of epigenetic regulation and its diverse applications (Phillips *et al*., 2019).

Future Prospect

Looking ahead, several promising developments are shaping the future of epigenetic research and applications. One exciting area of exploration is the role of 3D genome organization in gene regulation. Investigating how the spatial arrangement of chromosomes within the nucleus affects gene expression could lead to ground breaking insights into gene regulation mechanisms and new therapeutic strategies. Another emerging field is the interaction between the microbiome and the host epigenome. Recent research suggests that microbial communities may influence our epigenetic processes and, consequently, our health and susceptibility to diseases. This could pave the way for personalized medicine approaches where microbiome modifications are utilized to promote health and prevent illness. Technological advancements are also advancing the field. For example, single-cell epigenomics is set to revolutionize our ability to study epigenetic modifications at an unprecedented level of detail, enhancing our understanding of cellular diversity and function. High-throughput sequencing technologies continue to evolve, enabling more comprehensive and accurate epigenetic analyses. In the realm of therapeutics, new epigenetic drugs targeting non-coding RNAs and chromatin remodelers are on the horizon. These drugs promise more precise modulation of the epigenetic landscape, offering new treatment options for a variety of diseases. Combining epigenetic therapies with other therapeutic approaches, such as targeted therapies or immunotherapies, may further improve treatment outcomes and address complex conditions more effectively. As these technologies progress, it is essential to consider their ethical, legal, and social implications. Responsible use, equitable access, and the management of potential unintended consequences will be crucial for maximizing the benefits of epigenetic research.

The future of epigenetics holds great potential for advancing our understanding of gene regulation, improving disease treatments, and

personalizing medical care. The field continues to evolve with innovative technologies and approaches, promising exciting developments ahead.

Conclusion

The rapidly advancing field of epigenetics is poised to revolutionize our comprehension of gene regulation and disease mechanisms. Key areas of future exploration include the investigation of 3D genome organization and the influence of the microbiome on epigenetic processes. These research directions hold the potential to enhance our understanding of gene expression and pave the way for personalized medical approaches.

Technological advancements such as single-cell epigenomics and high-throughput sequencing are significantly improving our ability to study epigenetic modifications with greater accuracy. These innovations are likely to deepen our insight into cellular functions and variability. Emerging epigenetic drugs and their potential integration with other therapeutic strategies could offer new solutions for treating a variety of diseases more effectively. As these therapies develop, it will be important to address their ethical, legal, and social implications to ensure their responsible and fair application. The future of epigenetics promises to drive significant progress in scientific research, therapeutic development, and personalized medicine. As the field evolves, it is expected to reveal new opportunities and challenges, ultimately impacting both individual health and broader public health strategies.

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Chapter - 10
Review on the Antibacterial Activity of Phyto Extracts

Authors

Dipti Das
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Tanisha Bhowmick
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Tiyasha Saha
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Priyajit Banerjee
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Bidisha Ghosh
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Subhasis Sarkar
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Suranjana Sarka
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Chapter - 10

Review on the Antibacterial Activity of Phyto Extracts

Dipti Das, Tanisha Bhowmick, Tiyasha Saha, Priyajit Banerjee, Bidisha Ghosh, Subhasis Sarkar and Suranjana Sarkar

Abstract

The increasing prevalence of antibiotic resistance presents a significant challenge in managing bacterial infections, leading to the search for alternative treatment options. Nano phytoextracts, which integrate the antimicrobial properties of plant extracts with nanoparticle technology, represent a promising strategy to address resistant bacteria. This review evaluates the effectiveness of various Nano phytoextracts derived from medicinal plants, focusing on their mechanisms of action against bacterial pathogens. The combination of nanoparticles, such as silver, zinc oxide, and gold, with plant extracts enhances their antimicrobial activity and stability. Additionally, the formulations have the potential to reduce toxicity and improve bioavailability. The review also explores the challenges associated with the clinical application of nano phytoextracts and outlines future research directions. This comprehensive analysis highlights the promise of nano phytoextracts as an innovative approach in the battle against bacterial infections and stresses the importance of further investigation to maximize their therapeutic potential.

Keywords: Antimicrobial, bacterial infections, medicinal plants, nanoparticles, phytoextracts

Introduction

Antibiotics are crucial compounds that either eliminate bacteria (bactericidal effect) or inhibit their growth (bacteriostatic effect), playing an essential role in protecting the health of humans and animals. However, the overuse and misuse of antibiotics in humans and animals have raised concerns about the rise of antibiotic-resistant bacteria, a global health threat. The increase in antimicrobial resistance (AMR) among bacterial pathogens has contributed to higher rates of mortality and disability worldwide (Dugassa *et al*., 2017).

The emergence of multidrug-resistant Gram-positive and Gram-negative bacteria poses significant challenges in treatment and may render conventional therapies ineffective (Frieri *et al*., 2016). Plasmids, which are circular, double-stranded DNA molecules separate from the bacterial chromosome, often carry genes that confer antibiotic resistance, sometimes referred to as "jumping genes" (MacGowan *et al*., 2017). Even prior to the widespread availability of antibiotics, many bacterial species had already developed mechanisms of resistance. Antibiotic resistance can result from the uptake of external DNA or from mutations in the bacterium's existing genetic material, which may become permanent in infected individuals or animals (Larsson *et al*., 2021).

Treatment failures related to infections caused by resistant bacteria are influenced by various factors, including the bacteria's fitness, the severity of underlying health conditions, delays in initiating effective treatment, and sometimes the lack of appropriate therapies. Resistance genes do not always correlate with increased virulence but can enhance a pathogen's ability to survive and thrive. In clinical settings, the most resistant strains tend to be those that can persist in environments with high concentrations of antibiotics, making them more competitive than other strains within the same species. Notable "high-risk clones," such as K. pneumoniae ST258, E. coli ST131, Enterococcus faecium CC17, and P. aeruginosa ST235, are rapidly spreading and exhibit high levels of drug resistance, complicating treatment efforts. Resistance often leads to delays in effective therapy, partly due to discrepancies between initial empirical treatments and later antibiotic susceptibility testing.

In addition to bacterial resistance, toxins produced by phytoplankton represent a significant threat to humans, animals, and other organisms. Despite these dangers, phytoplankton toxins hold potential for the treatment of various diseases, including diabetes, AIDS, Alzheimer's disease, bacterial and fungal infections, schizophrenia, inflammation, allergies, osteoporosis, asthma, and pain. Ongoing research is focused on enhancing the clinical effectiveness of these toxins through the development of improved nano-formulations (Pradhan *et al*., 2021). Phytoplankton toxins show promise in fighting bacterial infections by interfering with quorum sensing and inhibiting biofilm formation. Bacterial biofilms, which are protected by an extracellular matrix and contain diffusion barriers, play a significant role in antimicrobial resistance by harboring persistent cells (Friedman *et al*., 2016).

Phytochemical-based adjuvants can efficiently penetrate bacterial biofilm barriers, thereby improving the efficacy of antibiotics and helping control the spread of antimicrobial resistance (AMR). Furthermore, phytochemicals can

target bacterial intracellular components, including degrading enzymes, efflux pumps, protein synthesis pathways, and DNA replication processes. In addition to pure phytochemicals, plant-derived nanomaterials have demonstrated great potential in overcoming bacterial biofilms. These nanoparticles can be engineered to penetrate biofilm structures and selectively target specific bacterial components.

This review explores the synergistic role of phytochemicals and plant-derived nanomaterials in preventing the progression of AMR associated with biofilms. It delves into the latest advancements and underlying mechanisms through which plant-based adjuvants and nanomaterials act to combat resistance (Kungwani *et al*., 2024).

Antimicrobial Resistance

Antimicrobial resistance (AMR) has emerged as a result of the increased and improper use of antibiotics. It refers to the ability of microorganisms-such as bacteria, viruses, fungi, and parasites-to withstand and multiply in the presence of drugs designed to eliminate them. Infections caused by antimicrobial-resistant organisms are not only difficult to treat, but they also pose a significant risk of severe illness and even death. Antimicrobial agents, including antibiotics, antifungals, antivirals, disinfectants, and food preservatives, work by either killing microorganisms or inhibiting their growth and reproduction (Salam *et al*., 2023).

Various natural, semi-synthetic, and synthetic antimicrobial agents function through distinct mechanisms to significantly disrupt microbial physiological and metabolic processes. For instance, β-lactams and glycopeptides interfere with cell wall synthesis, macrolides and tetracyclines block protein synthesis, sulfonamides inhibit metabolic pathways, and fluoroquinolones affect DNA replication and translation. Despite their effectiveness, the widespread and prolonged use of antibiotics has contributed to the development of AMR in bacteria (Abushaheen *et al*., 2020).

Factors Accelerating the Rate of Antimicrobial Resistance (AMR)

Misuse and Overuse of Antibiotics: A common issue contributing to antibiotic resistance is the inappropriate use of antibiotics, particularly their administration when they are not needed. In many low-income regions, such as Vietnam and India, the availability of substandard antibiotics over the counter is common due to weak enforcement of prescription regulations. This unrestricted access enables individuals to self-medicate for conditions where antibiotics are unnecessary. Additionally, doctors sometimes prescribe prolonged courses of antibiotics without sufficient evidence of their need,

which further promotes resistance. A significant driver of over prescription is financial incentives; for instance, some hospitals in China encourage doctors to prescribe antibiotics to receive greater compensation from pharmaceutical companies. Patient pressure also plays a role, as many patients expect antibiotics even for viral infections. Research indicates that one of the key challenges for doctors is the belief that patients expect antibiotic prescriptions (Dadgostar *et al*., 2019).

Biological Factors: Antibiotic resistance can arise from bacterial evolution and genetic mutations. Plasmids-small, circular DNA molecules found in bacteria-can acquire resistance genes through processes like transposons and insertion sequences. These plasmids can be transferred between different bacterial species, accelerating the spread of resistance. Horizontal gene transfer (HGT) between bacteria is another mechanism that promotes the dissemination of resistance genes, contributing to the global challenge of antimicrobial resistance (AMR) (Dadgostar *et al*., 2019).

Mechanisms of Antimicrobial Resistance: AMR mechanisms can be classified into four main types: limiting drug uptake, modifying drug targets, inactivating the drug, and active drug efflux. Gram-negative and Gram-positive bacteria utilize these mechanisms differently, primarily due to structural differences in their cell walls. Gram-negative bacteria can employ all four mechanisms, while Gram-positive bacteria are less likely to utilize drug uptake limitation and are unable to perform certain drug efflux processes.

- **Limiting Drug Uptake:** The lipopolysaccharide (LPS) layer in Gram-negative bacteria prevents certain antibiotics from entering the bacterial cell, granting them inherent resistance to some antibiotic classes. Mycobacteria, which have a lipid-rich outer membrane, allow hydrophobic drugs like rifampicin and fluoroquinolones to enter more easily, while hydrophilic drugs struggle. Antibiotics targeting the cell wall, such as β-lactams and glycopeptides, are ineffective against bacteria that lack a cell wall, like Mycoplasma, as they naturally resist these drugs. In contrast, Gram-positive bacteria generally face fewer barriers to drug penetration. However, certain Gram-positive bacteria like Staphylococcus aureus have developed resistance to vancomycin through mechanisms such as cell wall thickening, which provides moderate resistance and enables them to survive in the presence of the antibiotic, leading to the term VISA (vancomycin-intermediate Staphylococcus aureus).
- **Modification of Drug Targets:** One mechanism of resistance involves the modification of penicillin-binding proteins (PBPs) in

Gram-positive bacteria, particularly in response to β-lactam antibiotics. PBPs are enzymes involved in peptidoglycan synthesis in the bacterial cell wall. Alterations in PBPs, such as the reduction in the number of PBPs or the introduction of PBPs with a lower affinity for β-lactam drugs, can reduce the drug's effectiveness. In some cases, bacteria may acquire genes like mecA (in Staphylococcus aureus), which produces PBP2a, making it resistant to β-lactams.

- **Drug Inactivation:** Bacteria can neutralize antibiotics by breaking them down or by adding chemical groups that deactivate them. Enzymes like β-lactamases hydrolyze and inactivate β-lactam antibiotics, while other drugs, like tetracyclines, can be inactivated through hydrolysis by enzymes such as the tetX gene. Chemical modifications like acetylation, phosphorylation, or adenylation are common mechanisms for drug inactivation. For example, acetylation is an effective means of inactivating a variety of antibiotics, including aminoglycosides and fluoroquinolones.
- **Drug Efflux:** Efflux pumps are membrane transport proteins found on the bacterial chromosome that expel toxic substances, including antibiotics, from the bacterial cell. Many of these pumps are capable of expelling multiple drugs, a phenomenon known as multidrug resistance (MDR). Efflux pump expression can be induced or overexpressed in response to environmental conditions or the presence of certain drugs. High-level resistance often results from mutations that alter the efflux pump's structure, which improves its efficiency in expelling antibiotics. The capacity of efflux pumps can also depend on the availability of certain carbon sources to the bacteria (Reygaert *et al*., 2018).

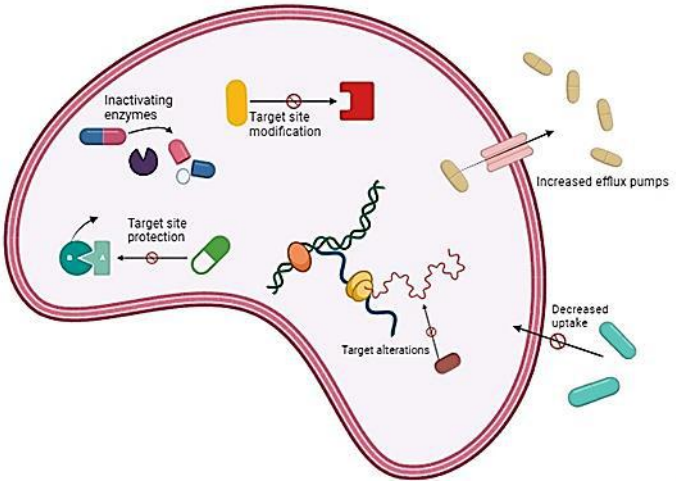

Fig 1: Mechanism of development antimicrobial resistance (Gaetano *et al*. 2023)

Impact of antimicrobial resistance on individual bacteria

Methicillin-resistant Staphylococcus aureus (MRSA) is a well-known example of antibiotic resistance, with significant implications for both healthcare costs and patient outcomes. Infections caused by MRSA result in longer hospital stays, increased diagnostic testing, and a greater need for medical and rehabilitative care, which drives up the overall treatment costs. MRSA shares many virulence factors with methicillin-susceptible Staphylococcus aureus (MSSA), including secreted proteins that aid in host cell invasion and surface molecules that enhance bacterial colonization. These factors allow the bacteria to cause a wide range of infections. MRSA, however, is particularly adept at spreading within healthcare settings, often leading to infections in the skin and surrounding tissues. Research indicates that MRSA infections have a mortality rate 2-3 times higher than infections caused by MSSA (Reygaert *et al*., 2018).

Phytochemical-based approaches for combating antimicrobial resistance

Phytochemicals offer promising potential in the fight against antimicrobial resistance due to their ability to target multiple bacterial molecular sites. While their binding affinity is typically lower than that of traditional antibiotics, which are designed to target specific bacterial molecules with high precision, phytochemicals' ability to interact with various bacterial components makes them a valuable tool in preventing resistance development. These plant-derived compounds can impact a range of bacterial structures, including the cell wall, cell membrane, and various proteins that

regulate vital bacterial functions. By interfering with processes such as metabolism and motility, phytochemicals can disrupt bacterial growth and function, providing a broad-spectrum approach to combatting bacterial infections (Díaz-Puertas *et al*., 2020).

Table 1: Phytochemicals and their antimicrobial effect

Phytochemicals	Plant	Antibacterial effect	Gram character	Ref.
Apigenin	*Petroselinum crispum, Matricariachamomilla, Apiumgraveolens, Basellarubra*	*Salmonella typhimurium, Enterobacteraerogenes, Proteus mirabilis*	Gram negative	Kashyap *et al*. 2018
Resveratrol	*Arachishypogaea, Vitisvinifer*	*Staphylococcus aureus, Campylobacter* sp., *Vibrio* sp., *E. coli*	Gram positive and Gram negative	Ma *et al*. 2018
Conessine	*Holarrhena floribunda, Holarrhenaantidysenterica, Funtumiaelastica*	*Pseudomonas aeruginosa*	Gram negative	Siriyong *et al*. 2017
Carvacrol	*Origanumvulgare*	*Staphylococcus aureus*	Gram positive	Silveira *et al*. 2020
Eugenol	*Syzygiumaromaticum, Eugenia caryophyllus*	*A. baumannii, Salmonella enteritidis, Campylobacter Jejuni, P. aeruginosa, E. coli*	Gram negative	Mac *et al* 2019
Quercetin	*Capparisspinosa, Polymnia fruticose, Ginkgo biloba*	*Salmonella enterica, Staphylococcus aureus, Pseudomonas aerugonisa*	Gram negative	Wang *et al*. 2018
Genistein	*Glycine max*	*Aeromonashydrophila*	Gram negative	Dong *et al*. 2021
Protocatechuic acid	*Scrophulariafrutescens*	*Yersinia enterocolitica*	Gram negative	Wu *et al*. 2022
Hydroquinone	*Vacciniummyrtillus*	*Pseudomonas aeruginosa, Staphylococcus aureus*	Gram negative and gram positive	Jeyanthi *et al*. 2021
Farnesol	*Vachelliafarnesiana*	*Listeria. Monocytogenes, Staphylococcus aureus*	Gram positive	Togashi *et al*. 2008
Rosthornin	*Rabdosiarosthornii*	*Propionibacterium acnes*	Gram positive	Tiwari *et al*. 2015
Ellagic acid	*Rosa rugosa*	*Helicobacter pylori*	Gram negative	De *et al*. 2018
Thymoquinone	*Nigella sativa*	*Shigellaflexneri*	Gram negative	Fan *et al*. 2021
Ursolic acid	*Malusdomestica*	*Klebseillapneumoniae*	Gram negative	Qian *et al*. 2020

Skullcapflavone II	*Scutellaria baicalensis*	*Mycobacterium aurum, Mycobacterium bovis, M. smegmatis*	Gram positive	Solnier *et al*. 2020
Arjunolic acid	*Syzygiumguineense, Syzygiumcordatum*	*Shigellasonnei*	Gram negative	Ghosh *et al*. 2013 Djoukeng *et al*. 2005
Asiatic acid	*Centellaasiatica*	*Clostridium difficile*	Gram positive	Harnvoravongchai *et al*. 2018
Artesunate	*Artemisia annua*	*Mycobacterium tuberculosis*	Gram positive	Qian *et al*. 2021
Capsaicin	*Piper nigrum, Capsicum annuum*	*Streptococcus pyogenes*	Gram positive	Marini *et al*. 2015 Agarwal *et al*. 2017
Fisetin	*Rhuscotinus*	*Mycobacterium bovis*	Gram positive	Brown *et al*. 2007
Norwogonin	*Scutellariabaicalensis*	*Acitenobacterbaumannii*	Gram negative	Miyasaki *et al*. 2013
Hibiscetin	*Hibiscus sabdariffa*	*Klebseilla pneumonia, Enterobacteraerogenes*	Gram negative	Djeussi *et al*. 2013
Curcumin	*Curcuma longa*	*Enterobacterfaecalis, P. aeruginosa*	Gram positive and Gram negative	Tyagi *et al*. 2015 Teow *et al*. 2016
Reserpine	*Rauvol fiaserpentina*	*Staphylococcus aurues*	Gram positive	Parai *et al*. 2020 Begum *et al*. 2012
Sakuranetin	*Polymniafruticosa*	*H. pylori*	Gram negative	Zhang *et al*. 2008
Betulinic acid	*Mikaniacordata*	*P. aeruginosa, S. aureus*	Gram negative and gram positive	Oloyede *et al*. 2017
Riccardin C derivatives	*Rebouliahemisphaerica*	*E. faecalis, P. aeruginosa, Vibrio parahaemolyticus*	Gram positive and gram negative	Morita *et al*. 2015
Rhein	*Rheum palmatum, Reynoutria japonica, Fallopia multiflora*	*Cutibacterium acnes*	Gram positive	Nguyen *et al*. 2020 Gong *et al*. 2019
Diosgenin	*Rhizomapolgonati, Smilax china, Trigonellafoenumgraecum*	*Porphyromonasgingivalis, Prevotella intermedia*	Gram negative	Cong *et al*. 2020

Andrographolide	*Andrographispaniculata*	*Burkholderiapseudomallei*	Gram negative	Zhang *et al*. 2020
Cinnamic acid	*Cinnamomum cassia*	*M. tuberculosis, Neisseria gonorrhoeae*	Gram positive and Gram negative	Guzman *et al*. 2014
Terminolic acid	*Syzygiumguineense*	*S. sonnei*	Gram negative	Ramos *et al*. 2021
Sulforaphane	*Brassica oleracea*	*H. pylori*	Gram negative	Fahey *et al*. 2013
Wogonin	*Agrimoniapilosa*	*P. aeruginosa*	Gram negative	Wang *et al*. 2021
Aloe-emodin	*Cassia occidentalis, Aloe vera, Polygonummultiflorum*	*S. aureus, Staphylococcus epidermidis, P. aeruginosa*	Gram positive and gram negative	Li *et al*. 2021
Punicalagin	*Punicagranatum*	*S. aureus*	Gram positive	Xu *et al*. 2017
Artemisinin	*Artemisia annua*	*S. aureus*	Gram positive	Appalasamy *et al*. 2014
Lonicerin	*Lonicera japonica*	*P. aeruginosa*	Gram negative	Xu *et al*. 2019
Morusin	*Morus alba*	*S. aureus*	Gram positive	Pang *et al*. 2019
Ursolic acid	*Malusdomestica*	*K. pneumoniae*	Gram negative	Qian *et al*. 2020
Berberine	*Berberis vulgaris, Berberisfremontii, Hydrastis Canadensis*	*H. pylori*	Gram negative	Huang *et al*. 2015
Epigallocatechin	*Camellia sinensis*	*P. aeruginosa, S. aureus*	Gram negative and gram positive	Bazzaz *et al*. 2016
Protocatechuic acid	*Scrophulariafrutescens*	*Yersinia enterocolitica*	Gram negative	Wu *et al*. 2022
Osthole	*Cnidiummonnieri, Angelica archangelica, Angelica pubescens*	*Salmonella typhimurium, K. pneumonia, A. baumannii*	Gram negative	Zhou *et al*. 2019
Taxifolin	*Silybummarianum, Allium cepa, Pseudotsugataxifolia, Pinuspinaster*	*E. faecalis*	Gram positive	Jeong *et al*. 2009
Chebulagic acid	*Terminalia chebula*	*A. baumannii*	Gram negative	Manso *et al*. 2021
Hexahydroxydiphenoyl	*Lythrumsalicaria*	*Proteus mirabilis, S. aureus*	Gram negative and	Becker *et al*. 2005

ester vescalagin			Gram positive	
Stigmasterol	*Neocaryamacrophylla*	*Streptococcus faecalis, S. aureus*	Gram positive	Mailafiya *et al*. 2018
Guggulsterone	*Commiphorawightii*	*E. coli, K. pneumoniae, P. aeruginosa, Salmonella typhi, E. faecalis, S. aureus*	Gram negative and gram positive	Jain *et al*. 2013

Curcumin as an antibacterial agent

Curcumin, the primary active compound in turmeric, is a hydrophobic mixture composed of three curcuminoids in the following proportions: 17% demethoxycurcumin (DMC), 3% bisdemethoxycurcumin (BDMC), and 77% curcumin. Turmeric, derived from Curcuma longa, belongs to the ginger family and is primarily cultivated in tropical regions of southern and southwestern Asia (Kocaadam *et al*., 2017). Curcumin has shown significant antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, such as Salmonella enterica serotype Typhimurium, Streptococcus mutans, Listeria innocua, Streptococcus pyogenes, Staphylococcus aureus, Helicobacter pylori, Acinetobacter baumannii, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus epidermidis, and Bacillus cereus. It also exhibits antibacterial effects against multidrug-resistant strains, including MRSA and Klebsiella pneumoniae resistant to polymyxin (Taghavifar *et al*., 2022).

Mechanisms of Action

Cell Membrane Disruption

Curcumin can integrate into bacterial cell membranes, disrupting their permeability and integrity, leading to bacterial cell death (Varshney *et al*., 2013). Its lipophilic nature allows it to penetrate bilayers, particularly affecting 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) membranes and interfering with processes such as exocytosis and membrane fusion (Barry *et al*., 2009). This ability to disrupt bacterial membranes likely contributes to curcumin's bactericidal activity, and it may also enhance membrane permeability, facilitating the uptake of other drugs (Zheng *et al*., 2020).

Suppression of Cell Division

The protein filament temperature-sensitive Z (FtsZ) is critical for bacterial cell division, and curcumin has been shown to directly interact with FtsZ in bacteria such as Bacillus subtilis and Escherichia coli, inhibiting the formation of the cytokinetic Z ring. It also enhances the GTPase activity of FtsZ, preventing its polymerization. Molecular docking studies indicate that curcumin binds to the GTPase catalytic pocket of FtsZ, disrupting the formation of the Z ring (Kaur *et al*., 2010).

Activation of Reactive Oxygen Species (ROS)

At minimum inhibitory concentrations (MICs), curcumin induces the production of reactive oxygen species (ROS) in bacterial cells, which leads to

cell death. This includes the generation of superoxide anions (O2•−), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH•), which damage bacterial cell components such as DNA, proteins, and membrane lipids. Curcumin also induces apoptosis-like responses in E. coli, including ROS accumulation, membrane depolarization, and increased Ca2+ influx (Zheng *et al*., 2020). Additionally, curcumin inhibits the bacterial SOS response, which is critical for DNA repair and biofilm formation (Butala *et al*., 2009).

Disruption of Bacterial Metabolism

Curcumin influences bacterial metabolism by increasing the production of NADH and ROS, both of which contribute to bacterial cell death. In S. aureus, curcumin treatment affects the levels of metabolites such as nitric oxide, kynurenine, and total thiol, indicating metabolic disruption. The activation of the kynurenine pathway reduces the availability of essential nutrients like L-tryptophan, limiting bacterial growth (Adeyemi *et al*., 2020).

Regulation of Intracellular Bacterial Propagation

Pre-treatment of macrophages with curcumin reduces intracellular infections caused by Shigella flexneri and Listeria monocytogenes. However, curcumin aggravates infections caused by Salmonella enterica serovar Typhimurium, S. aureus, and Yersinia enterocolitica, possibly because these bacteria have developed mechanisms to prevent pathogen-containing vacuoles from fusing with lysosomes. Conversely, Listeria monocytogenes and S. flexneri can escape into the cytosol, avoiding lysosomal degradation (Marathe *et al*., 2012; Bai *et al*., 2016). Curcumin also protects macrophages from Mycobacterium tuberculosis infection by inducing apoptosis, autophagy, and activating nuclear factor-kappa B (NF-κB) signaling pathways.

Phytotoxicity and Photodynamic Therapy

Curcumin also acts as a photosensitizer for photodynamic therapy. It absorbs blue light in the range of 455 to 460 nm, and upon activation, generates ROS that damage bacterial membranes, proteins, and DNA, leading to cell death. Studies suggest that curcumin can be used as a coating for medical devices like endotracheal tubes, helping to prevent ventilator-associated pneumonia by inhibiting bacteria such as E. coli, P. aeruginosa, and S. aureus through photodynamic inactivation when exposed to blue light (Huang *et al*., 2020; Chen *et al*., 2021).

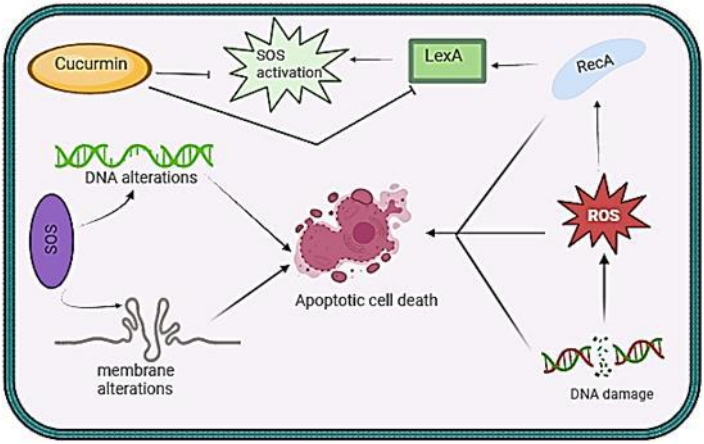

Fig 2: Antimicrobial activity of cucurmin (Dai *et al*. 2022)

Market Value of Medicinal Plant Extracts

The investigation of the phytochemical profiles of plants has gained increasing importance in the realms of both medicinal and food plant research. Plants produce secondary metabolites, such as phenolics, terpenes, and organic acids, primarily as a defense mechanism against environmental stressors (Savickiene *et al*., 2024). Despite the widespread approval of synthetic antimicrobial agents in many countries, plant-derived natural substances continue to attract significant research attention (Moloney *et al*., 2016). Medicinal plants represent a promising source of bioactive compounds capable of combating resistant bacteria (Verpoorte *et al*., 1998; Mahmood *et al*., 2013). The wide array of natural chemicals found in medicinal plants holds potential for enhancing the effectiveness of existing antibiotics, thus contributing to the fight against resistance (Lewis *et al*., 2006).

Phytochemicals, which are secondary metabolites produced by plants for medicinal purposes, form a crucial area of research. These compounds, either intermediate or final products of secondary plant metabolism, have been the focus of significant interest in recent decades, particularly in the context of developing alternative and complementary treatments to address antimicrobial resistance (Alsheik *et al*., 2020). It is believed that many bioactive compounds in natural sources, especially within plants (Kingdom Plantae), remain undiscovered (van Buren *et al*., 2011). Plant extracts, rich in phytochemicals like polyphenols, alkaloids, and terpenoids, have demonstrated antibacterial properties, including activity against antibiotic-resistant strains (Álvarez-Martínez *et al*., 2021).

Overview of the market size

The global plant extract market was valued at approximately USD 34.4 billion in 2022 and is projected to grow at a compound annual growth rate (CAGR) of 12.3%, reaching USD 61.5 billion by 2027. This growth is attributed to several factors, including the rising consumer demand for natural and health-oriented products, advancements in extraction technologies, and the discovery of new plant species with beneficial properties. As urban populations grow more health-conscious, there is an increasing preference for alternative medicines and therapies, leading to greater reliance on natural products. The ongoing digital transformation also plays a role in this expansion, providing solutions to meet the increasing demand (Jubair *et al*., 2021).

In the Asia-Pacific (APAC) region, market growth is driven by the growing popularity of vegan products and the incorporation of plant extracts as ingredients. According to the Botanical Survey of India, over 8,000 species of medicinal plants are found in India. However, some of these species are threatened by factors like forest degradation and extinction. The supply of such plants is limited due to their restricted cultivation areas, and many species only produce once a year, resulting in supply-demand imbalances. These challenges, along with the high costs of raw materials and storage, contribute to price variations and make it difficult to standardize product pricing (Van *et al*., 2011).

Challenges in Medicinal Plant Antimicrobial Activity

While medicinal plant extracts offer promising natural alternatives to synthetic antimicrobials, their current potential as antibacterial agents is not fully realized. Though these natural substances are widely available, affordable, and simple to use, the proportion of licensed antibacterial agents derived from medicinal plants remains low. Despite their potential, plant-based natural extracts have limitations when used as antibacterial treatments. It is crucial to approach medicinal plant ingredients with caution due to the lack of extensive evidence proving their effectiveness. Many studies remain inconclusive, and well-controlled, double-blind clinical and toxicological trials demonstrating the safety and efficacy of these extracts are still rare (Calixto *et al*., 2000).

Furthermore, issues such as adulteration, inconsistent cultivation and harvesting practices, lack of standardization, and inadequate storage conditions pose obstacles to the discovery of new antimicrobial agents from medicinal plants (Nijume *et al*., 2012). Factors such as harvest season, plant

growth location, the specific plant parts used, and processing methods can all influence the concentration and activity of active compounds in plant extracts. Additionally, local climate and environmental conditions affect the composition of plant extracts, making it challenging to standardize and compare antibacterial data across various studies (Radulović *et al*., 2013).

Challenges in developing new antimicrobials from medicinal plants

Research into new plant extracts faces substantial challenges due to the complexity and diversity of the compounds involved. Plant extracts contain numerous chemicals in varying quantities, making it difficult to identify the specific compounds responsible for a given biological effect. Overcoming these challenges is essential for developing new antimicrobials that can help address the growing issue of antibiotic resistance. One of the primary hurdles in creating new antibiotics is translating findings from in vitro studies to in vivo experiments and, ultimately, human clinical trials. In vivo research is necessary to understand the precise mechanisms of plant-derived compounds and assess their potential as alternatives or supplements to existing treatments for microbial infections.

Additionally, assessing plant extracts for their minimum bactericidal concentration (MBC) is crucial in evaluating their bactericidal properties and potential to reduce antimicrobial resistance. Integrating traditional knowledge about medicinal plants presents great potential for creating biocompatible solutions and accelerating the discovery of new antibiotics. Such knowledge is key to developing effective, eco-friendly extraction techniques and maximizing the benefits of bioactive plant extracts. Standardizing the extraction process and in vitro testing methodologies can make the search for new antimicrobial agents more systematic, leading to more accurate research results. However, limited research funding has impeded progress in studying medicinal plants, resulting in a shortage of high-quality studies on the structure-activity relationships of specific compounds. Despite these challenges, there remains a strong interest in developing new antimicrobials from traditional plant medicines, as addressing antimicrobial resistance demands the creation of more affordable and effective antibiotics.

Future prospects

The bioactive compounds found in medicinal plants remain largely untapped and represent a largely unexplored resource. Many plant species yet to be studied hold great promise for discovering compounds capable of modifying resistance mechanisms and serving as potential therapeutic agents (Quave *et al*., 2016). Future research should focus on modifying these

compounds to improve their pharmacokinetics and pharmacodynamics, as well as understanding their structure-activity relationships. Further studies should also investigate the synergistic effects between medicinal plant extracts and antibiotics, considering not only their antimicrobial activity but also their ability to target various biological pathways. Understanding the interactions between plant extracts and antimicrobial drugs is crucial for identifying synergistic or antagonistic effects.

More comprehensive investigations, including in vivo studies and toxicity testing, are necessary to evaluate these compounds as biological agents. Studies should also assess the minimum inhibitory concentration (MIC) of plant extracts to facilitate direct comparisons with current antibiotics. Enhancements in in vitro antimicrobial susceptibility testing (ASTs) and the development of validated in vivo ASTs are crucial. Improving the efficiency of ASTs does not necessarily require the introduction of complex new techniques but rather the adoption of best practices across the entire production and supply chain for medicinal plant-based products.

Modern biotechnological tools, such as genomics, proteomics, and metabolomics, are increasingly applied to medicinal plant research, propelling the discovery of alternative natural antimicrobials. Integrated technologies capable of identifying active compounds, characterizing their interactions, and elucidating their mechanisms of action simultaneously are still in development. As the demand for safe and effective plant-derived products continues to rise, there is an increasing need for innovative screening methods and robust quantitative data to assess their activity. To accurately evaluate and compare research findings, scientific organizations must establish standardized technical guidelines for analyzing medicinal plant extracts. Collaborations between resource-rich institutions and biodiversity-rich areas will advance medicinal plant research by sharing expertise and offering research training opportunities for students and academics (Nagora *et al*., 2011).

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